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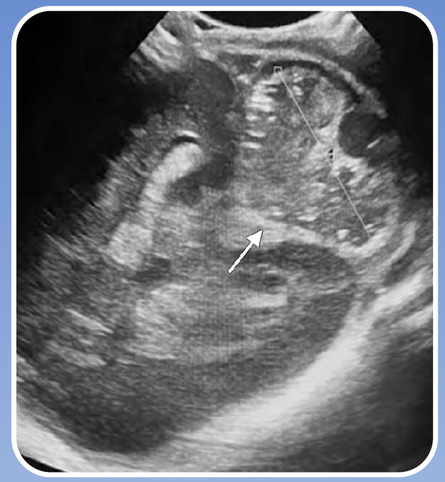
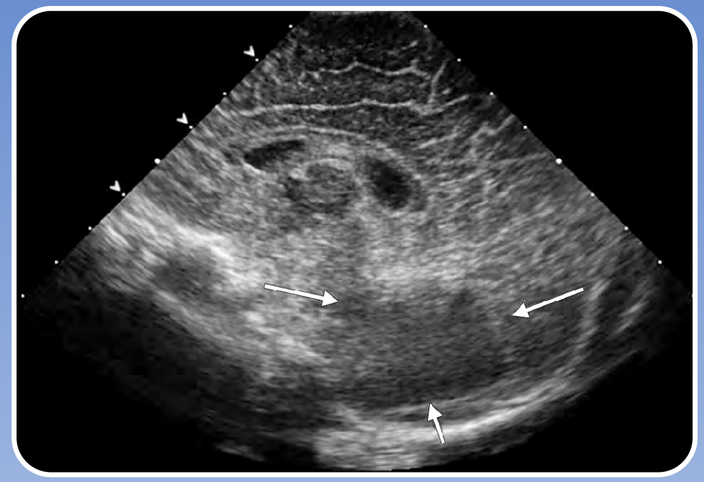


Sonographic image (right mastoid foramen) shows cerebellum (right upper corner; arrow)



Sonographic image (sagittal midline) shows a small remnant of the cerebellum overlying a large cystic Dandy-Walker malformation (marked by arrows)

Other highlighted articles:

Evaluation of a Cranial Ultrasound Scoring System for Prediction of Abnormal Early Neurodevelopment in Preterm Infants

Linked Th17 and Calgranulin Responses in Maternal-Cord Blood Dyads of Preterm Gestations with Histologic Chorioamnionitis

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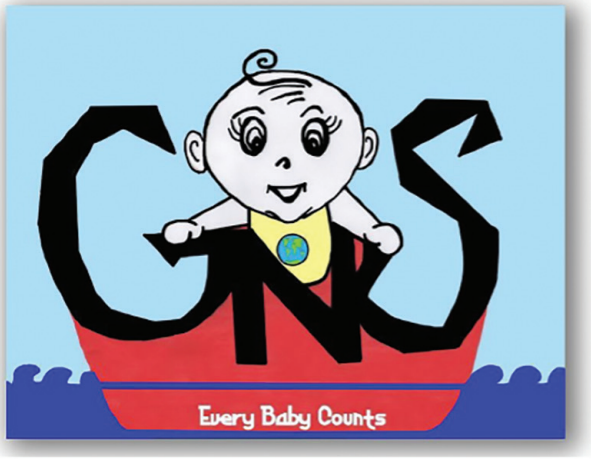

Global Newborn Society

Each time we lose an infant, we lose an entire life and its potential!

Newborn is the official journal of the Global Newborn Society (GNS), a globally-active, non-profit organization that is registered as a 501(c)(3) non-profit formation in the United States and is currently being listed as an analogous charity in many other nations. The aim is to enhance research in newborn medicine, understand epidemiology (risk-factors) of disease, train healthcare workers, and promote social engagement. The GNS was needed because despite all improvements in medical care, infants remain a high-risk patient population with mortality rates similar to 60-year-olds. We need to remind ourselves that *Every Baby Counts*, and that *Each Time We Lose an Infant, We Lose an Entire Life and its Potential*.

Our logo above, a hand-drawn painting, graphically summarizes our thought-process. There is a lovable little young infant exuding innocent, genuine happiness. The curly hair, shape of the eyes, long eye-lashes, and the absence of skin color emphasize that infants need care all over the world, irrespective of ethnicity, race, and gender. On the bib, the yellow background reflects happiness, hope, and spontaneity; the globe symbolizes well-coordinated, world-wide efforts. The age-related vulnerability of an infant, with all the limitations in verbal expression, is seen in being alone in the boat.

The unexpressed loneliness that many infants have to endure is seen in the rough waters and the surrounding large, featureless sky. However, the shades of blue indicate that the hope of peace and tranquility is not completely lost yet. The acronym letters, GNS, on the starboard are made of casted metal and are pillars of strength. However, the angular rough edges need continued polishing to ascertain adequacy and progress. The red color of the boat symbolizes our affection. The expression "*Every Baby Counts*" seen on the boat's draft below the waterline indicates our commitment to philanthropy, and if needed, to altruism that does not always need to be visible. The shadow behind the picture shows that it has been glued on a solid wall, one built out of our adoption and commitment.

Design of the Journal Cover

The blue color on the journal cover was a careful choice. Blue is the color of flowing water, and symbolizes the abnormalities of blood vascular flow that are seen in many neonatal illnesses. There is a gradual transition in the shades of blue from the top of the cover downwards. The deeper shades of blue on the top emphasize the depth, expertise, and stability, which the renowned authors bring. Light blue is associated with health, healing, tranquility, understanding, and softness, which their studies bring. The small letter "*n*" in the title of the journal, *newborn*, was chosen to emphasize the little size of a newborn baby. The issue editors chose three articles to be specifically highlighted; the two pictures and two titles below reflects an order suggested by them.

Instructions to Authors

The journal welcomes original articles and review articles. We also welcome consensus statements, guidelines, trials methodology, and core outcomes relevant to fetuses/young infants in the first 1000 days. A detailed set of instructions to authors can be seen at https://www.globalnewbornsociety.org/instructions-for-authors. The manuscripts can be submitted online.

Issue Information

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Contents

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We Need New Tools to Evaluate Neurological Development *in Utero* and after Birth

Fetuses, newborns, and young infants are highly susceptible to neurological injury.[1,2] Damage to primordial structures during early development can result in malformations.[3–5] Later, injuries can disrupt many of these basic structures in the growth phase.[6,7] Many infectious and non-infectious stimuli can trigger inflammation with its changes, including vasomotor dysregulation with edema and temperature instability, and leukocytosis.[8,9] Our ability to restore damaged neurological structures is still limited, and therefore, the emphasis remains on early detection by cranial imaging and supportive measures.[4,10,11]

Similar to diseases affecting other organs, the debate continues about the relative contribution of infectious agents, vasomotor changes, and immaturity of the immune system in the pathogenesis of various neurodevelopmental disorders.[12] Many infectious agents that affect the fetus *in utero* or during early infancy cannot be treated in a timely fashion.[13] Many drugs still need evaluation, and some that are currently in use have limited efficacy.[14] Others have had unacceptable short- and long-term adverse effects.[15] To appropriately tailor these treatments and minimize risk, accurate neuroimaging is important for early detection of pathogen-induced and other inflammatory changes.[16,17] If we can understand the temporal evolution of these changes, we might be able to make a difference. There is a need for monitoring paradigms and new treatments. All treatment modalities are not uniformly available or affordable in different parts of the world, and hence there is a need for computational systems to assess, monitor, and treat these highly susceptible patients.[18] If we know the possibilities, we can educate and motivate our care providers to acquire and learn these tools.[19]

Our journal, the *Newborn* aims to cover fetal/neonatal problems that begin during pregnancy or occur after birth during the first 1000 days after birth. In this 2nd issue of the second volume, we present 8 important articles **(Figure 1)**. In an original study, McLean et al.[20] evaluated a cranial ultrasound scoring system for prediction of abnormal early neurodevelopment in preterm infants. In a retrospective, single-center study, they studied cranial ultrasound scans of 242 preterm infants at a chronological age of 6 weeks to compare this scoring system to conventional sonographic detection of abnormalities such as intracranial hemorrhages, white matter lesions, and cystic periventricular leukomalacia.[21–23] The aim was to determine whether the scoring system could enhance our accuracy in predicting developmental delay or cerebral palsy (CP) in preterm infants.[24,25] They did not find any differences in sensitivity/specificity[26] when the entire cohort was studied. However, in the subset with severe cranial ultrasound abnormalities, the cUS scores showed higher sensitivity (57% vs. 27%, [95% CI: 12 to 49]) but lower specificity (68% vs. 96%, [95% CI: -21 to -34]) for predicting CP. Similarly, there was higher sensitivity (44% vs. 12% [95% CI: 23 to 41]) but lower specificity (74% vs. 98%, [95% CI: -15 to -32]) for developmental delay. These newer methods of clinical screening can help in prioritization, use of specific neuroimaging protocols and laboratory investigations, tailoring of therapeutic methods, and the frequency and goals of follow-up.[27]

There are two important studies in this issue that focused on necrotizing enterocolitis and chorioamnionitis, respectively. In addition to local effects in the damaged organ systems, both these conditions are known to affect neurodevelopmental outcomes.[28–34] In the first, Rothers et al.[35] compared patients with necrotizing enterocolitis (NEC) and controls and evaluated the impact of enteral feeding and antibiotic treatment on stool total bile acid (TBA) content. Accumulation of ileal bile acids is a crucial component of NEC pathophysiology; infants who develop NEC show high coefficients of variation of TBAs (CV-TBAs).[36,37] High values for CV-TBA levels predicted NEC status among infants, but the type of feeds and antibiotic usage did not drive this relationship. In the second study, Buchanan and colleagues[38] examined Th17 and calgranulin responses in maternal-cord blood dyads of preterm gestations with histologic chorioamnionitis.[39] Our understanding of the Th17 responses in chorioamnionitis is relatively limited.[40] The authors have examined Th17 responses[41] in 47 maternal–cord blood dyads of preterm gestations,[42] for Th17-linked cell frequencies and plasma calgranulin (S100A8, S100A12).[43–45] In those with fetal inflammation, there was increased frequency of Th17 cells and plasma levels of calgranulin.[46,47] Cord blood S100A12

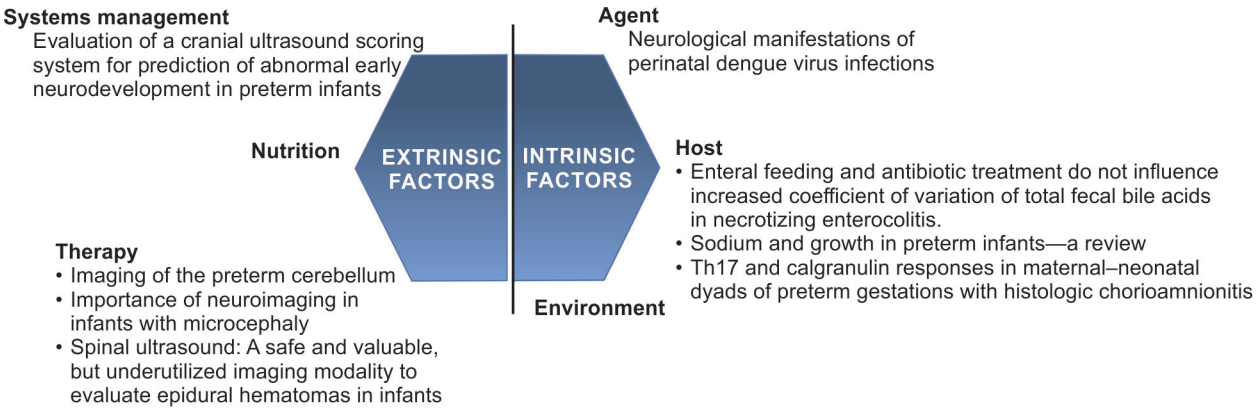

Fig. 1: **Areas of focus in the *Newborn*, volume 2, issue 2.** The *Newborn* has expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health; these originate in therapy, nutrition, and systems management. In volume 2, issue 2, we cover 4 of these foci, namely infectious diseases, host factors, therapy, and systems management.

levels correlated with Th17 cell frequencies.[48] These data are interesting and are likely to evoke further examination in larger samples and in appropriate animal models.[49]

This issue carries considerable information on emerging goals and methods of neurological imaging in premature and critically ill infants. Rangwani and her colleagues[50] have provided an overview of the overall evaluation of microcephaly, a head (occipitofrontal) circumference that is 2 standard deviations or lesser than average, accounting for age and gender.[51,52] We can now use neuroimaging to enhance current methods of clinical evaluation. They have described the implications of altered brain volume, the size and shape of the skull, and the timing of onset of these abnormalities.[17] There is a clear need for a multifaceted approach. In another article, Kalamdani and coworkers[53] have focused on the role of medical imaging in the assessment of cerebellar injury in preterm infants. The cerebellum continues to grow during the 3rd trimester and is at a higher risk of structural abnormalities resulting from altered formation or partial destruction.[54] The authors have summarized the advances in posterior fossa imaging and the appearance of various abnormalities on cranial ultrasound and high-resolution anatomical and functional magnetic resonance imaging (MRI). The role of advanced MRI modalities like functional MRI,[55] diffusion tensor imaging,[56] and MR spectroscopy[57] are also discussed in some detail. In another article, Ogu et al.[58] have described a series of cases where they used spinal ultrasound to evaluate epidural hematomas.[59] The accessibility, cost-effectiveness, and accuracy of spinal ultrasound make it an appealing alternative to MRI.[60] They have discussed the advantages of incorporating spinal ultrasound into clinical practice for timely and convenient diagnosis of spinal epidural hematomas in neonates and young infants.

Araya et al.[61] have reviewed sodium homeostasis in neonates. In premature and critically ill term infants, sodium depletion has important implications in extrauterine growth restriction and cardiometabolic and neurodevelopmental disorders.[62] There are compelling data from animal models, which still need to be confirmed in human subjects. The authors aim to increase the awareness of sodium homeostasis in preterm infants and have provided sodium intake recommendations based on currently available literature.

Finally, Singh and coworkers[63] have described neurological manifestations of perinatal dengue. Dengue viruses are single-stranded RNA viruses; these are mosquito-borne human pathogens seen in periequitorial and tropical regions.[64,65] Mother-to-fetus transmission of the virus leads to congenital dengue disease.[66] These should be suspected in endemic regions in infants with fever, a maculopapular rash, and thrombocytopenia. Neurological manifestations include intracerebral hemorrhages, neurological malformations, and acute focal/disseminated encephalitis/encephalomyelitis.[67] We do not have proven specific therapies yet; supportive management is focused on close monitoring and maintenance of intravascular volumes.

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ORIGINAL RESEARCH

Imaging of the Preterm Cerebellum

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Abstract

Cerebellar injury is being increasingly recognized as a significant complication of preterm birth. A critical phase of cerebellar growth occurs during the third trimester characterized by cellular migration, proliferation, and arborization. This vulnerable developmental phase increases the risk of impaired cerebellar development, especially in preterm infants, given their exposure to adverse extrauterine environments. Cerebellar malformations and disruptions are the types of cerebellar insults encountered. A "malformation" is defined as a non-progressive, congenital morphologic anomaly of a single organ or body part following altered primary development. A "disruption" is defined as a non-progressive, congenital morphologic anomaly following the breakdown of a body structure that had the normal potential for development. Advances in neonatal neuroimaging with increased use of mastoidal and suboccipital views focusing on the posterior fossa by cranial ultrasound (cUS) and high-resolution anatomical and functional magnetic resonance imaging (MRI) have improved the sensitive and specific identification of posterior fossa abnormalities, in particular of cerebellar injury in preterm neonates. This article discusses the various modalities of neuroimaging of the cerebellum with advantages and disadvantages. Ultrasonography (USG) is the most easily available and feasible bedside modality of imaging, though it has the disadvantage of not detecting subtle abnormalities like punctate hemorrhages. Conventional T1 and T2 weighted MRI can detect most of the cerebellar malformations and disruptions in preterm infants. But the logistics of MRI at most institutions make it less feasible during the first few weeks of life for extremely preterm neonates. The role of advanced MRI modalities such as functional MRI, diffusion tensor imaging (DTI), and magnetic resonance (MR) spectroscopy in cerebellar disruptions and malformations are also discussed in some detail.

Keywords: Cerebellar hemorrhage, Cerebellum, Diagnostic imaging, Disruptions, Magnetic resonance imaging, Malformations.

Newborn (2023): 10.5005/jp-journals-11002-0061

Background

Cerebellar injury is being increasingly recognized as a significant complication of preterm birth. A critical phase of cerebellar growth occurs during the third trimester characterized by cellular migration, proliferation, and arborization.[1,2] This vulnerable developmental phase increases the risk of impaired cerebellar development, especially in preterm infants, given their exposure to adverse extrauterine environment.[3]

Advances in neonatal neuroimaging with increased use of mastoidal and suboccipital views focusing on the posterior fossa by cranial ultrasound (cUS) and high-resolution anatomical and functional magnetic resonance imaging (MRI) have improved the sensitive and specific identification of posterior fossa abnormalities, particularly in cerebellar injury in preterm neonates. Up to 19% of very preterm (VP: gestation at birth <32 weeks) have a cerebellar injury on MRI, and these rates are higher in those with a birth weight below 750 gm.[4]

Impaired cerebellar development occurs by the following three mechanisms: (A) Direct cerebellar injury, such as cerebellar hemorrhage (CBH) causing tissue loss, atrophy, and subsequent growth failure (and consequently, permanent cerebellar disruption); (B) indirect cerebellar injury or cerebellar disruption (underdevelopment) with or secondary to supratentorial cerebral injury. Reduced blood flow and altered metabolism in the contralateral CBH may cause some localized ischemia and result in a migration anomaly and a cerebellar cleft, a phenomenon that has been described as "crossed cerebro–cerebellar diaschisis" with hypoplasia and impaired cerebellar growth); and (C) cerebellar underdevelopment in the absence of direct cerebellar or cerebral

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injury, as in malformations. Examples of such an anomalous, often genetically encoded abnormal "anlage" of the cerebellum can be seen in Dandy–Walker malformations, prematurity-related factors such as altered placental growth, genetic/chromosomal anomalies, and compromised immature cerebral/systemic circulation.[5]

The cerebellum has long been known for its essential functions in motor learning and coordination.[6] Besides this well-acclaimed role, there has been increasing appreciation of its importance in neurocognitive functions, including cognition, behavior, language, memory, and learning.[7] There are dynamic, closed-loop circuits between the cerebellum and the cerebral cortex, and are essential for the structural and functional development of the distal cortical regions to which these projects.[8] In preterm infants, cerebellar injury has a profound impact on the early development of these neural circuits in a location-dependent manner;[9] disruption of certain cerebro–cerebellar circuits has been associated with altered neurodevelopment[10] such as in autism spectrum disorders (ASDs),[11] language impairment,[12] and attention deficit and hyperactivity disorders (ADHD).[13]

Cerebellar development begins during the early embryonic period,[7] from around 4 weeks of gestation and continues into the early postnatal years.[14] The third trimester of pregnancy is a critical period for cerebellar development; there is at least a 5-fold expansion in volume[15,16] and a 30-fold increase in its surface area.[16,17] These changes have been attributed to the proliferation and differentiation of the external granule precursor cells.[18] Cerebellar histogenesis is a complex process determined by a large number of genes, and may be summarized in the following four fundamental steps: (A) Characterization of the cerebellar territory in the hindbrain; (B) differentiation of two actively proliferating cellular clusters, the Purkinje and granule cells; (C) inward migration of granule cells; and (D) differentiation of cerebellar neurons. This rapid growth renders the developing cerebellum particularly vulnerable to various insults and consequent disruption of its complex, programmed developmental course.[19] These may result in (A) malformations (primary abnormalities), defined as non-progressive, congenital morphologic anomalies occurring due to altered primary development; and (B) disruptions, which are non-progressive, congenital morphologic anomalies resulting from structural breakdown of developing regions that had normal potential for development. Disruptions typically occur in prenatal life following a single or a sequence of multiple events that disrupt normal growth and development of the cerebellum. Some potential causes of disruption include vascular, infectious, teratogenic, and mechanical. It is important to differentiate between cerebellar disruptions and malformations from the perspective of diagnosis, prognosis, treatment, and genetic/parental counseling. The preterm cerebellum is predisposed to both disruptions and malformations.

Imaging of the Cerebellum

Cranial Ultrasound

Cranial Ultrasound is the most feasible modality for neuroimaging in high-risk preterm infants, particular in the first few weeks of life. Anterior fontanelle (AF) is commonly used as the acoustic window to look at the supratentorial structures and lesions (Fig. 1). However, the longer distance between the transducer and posterior fossa structures causes some limitations. Mastoidal fontanelle (MF) provides a better acoustic window to image the brainstem and cerebellum. The MF is located at the junction of the parietal, temporal, and occipital bones (Fig. 2). While performing the examination, a transducer with a small footprint is placed over the MF, behind the helix of the ear. This reduces the distance between the transducer and cerebellum and allows the use of higher frequency transducers (8–15 MHz) with better-quality images.[20–22] The posterior fontanelle (PF) is also useful to obtain posterior fossa images. Coronal sections obtained through PF show the fourth ventricle choroid plexus; cerebellar hemispheres; the brainstem; the occipital horn and trigonum of the lateral ventricles; the occipital lobes; and overlying subarachnoid spaces and tentorium cerebelli. These views are useful for the detection of CBH.[23] The trans-nuchal acoustic window is increasingly recognized as a feasible alternative to visualize the cerebellum in VP infants.[24]

Cerebellar hemorrhage can occur in high-risk preterm neonates during the first postnatal week after birth, at the same time as the germinal matrix hemorrhage and intraventricular hemorrhage (GMH-IVH). Most CBHs occur in gray matter (zones 1 and 2), which develops from the germinal cell layers of the rhombic lip during embryogenesis; the exclusive involvement of white matter is uncommon.[25,26] Three patterns of CBH have been described as follows: (A) Punctate (≤4 mm); (B) limited (>4 mm but less than one-third of the cerebellar hemisphere[s]); and large (more than or equal to one-third of the cerebellar hemisphere[s]) (Fig. 3).[25] Cerebellar hemorrhage can be uni- or bilateral and either symmetric or asymmetric. The involvement of the vermis, which is isolated or associated with cerebellar hemispheric lesions, is noted separately. Overall, the severity of CBH is inversely proportional to the gestational age of the infant. Punctate CBH (<4 mm) is usually detected on MRI and is not readily seen by cUS even in MF views.

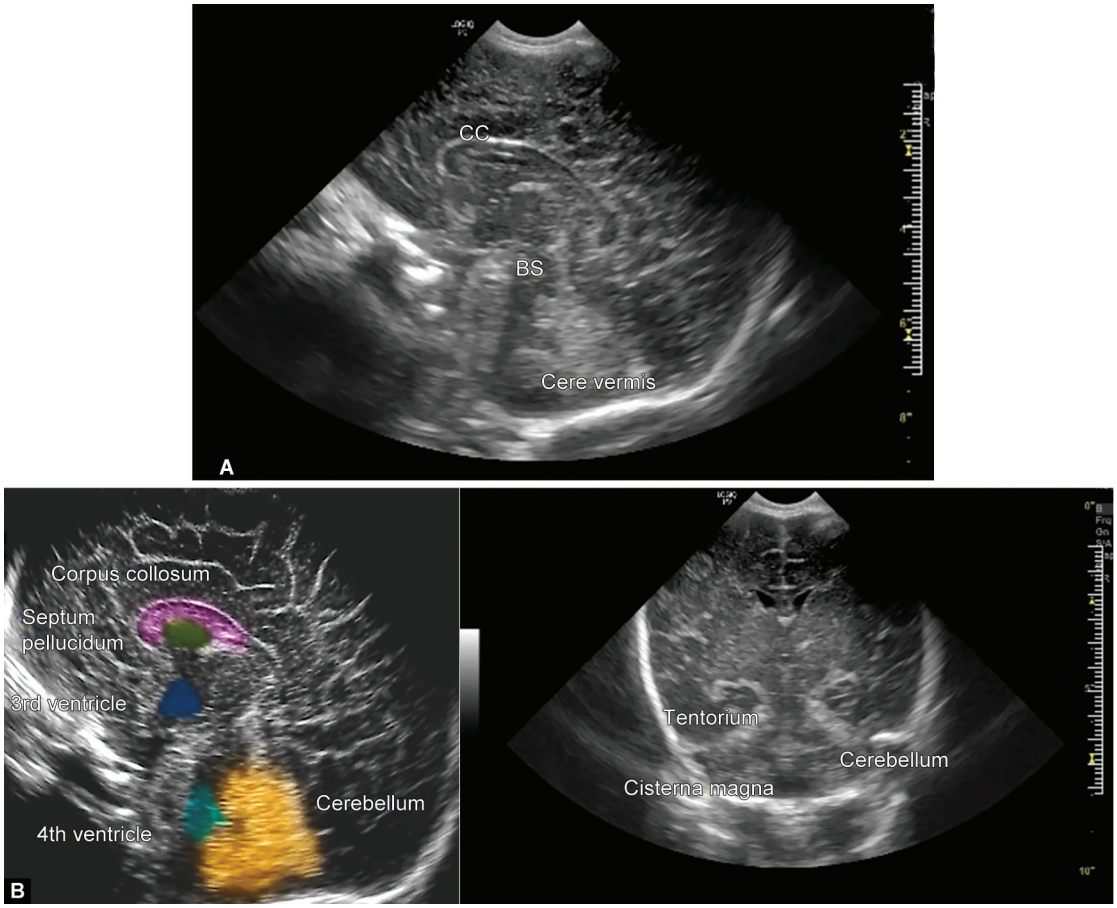
Impaired cerebellar development can occur as an isolated anomaly and not be associated with hemorrhage(s). There is low cerebellar volume, and it has been associated with subnormal neurodevelopmental outcomes.[7] The transverse cerebellar diameter is used to quantify cerebellar volume on ultrasonography (USG). It can be measured in the MF view in both planes (Fig. 4). Gestational age-specific nomograms are available to compare the measurements. Cranial ultrasound is also useful for recognizing posterior fossa anomalies, such as the Dandy–Walker malformation (Fig. 5A).

Cerebellar infarction has been reported in extremely preterm infants or as a part of hypoxia–ischemia-mediated injury in near term and term infants. In acute stages, cUS may show a diffusely increased echogenicity but if these findings persist, an MRI would be useful for confirmation. Subsequently, at around term–equivalent age, it may manifest as cerebellar atrophy or severe volume loss on cUS.

The role of volumetric analysis of different brain parts (whole brain, thalamus, frontal cortex, and cerebellum) using 3D-cUS in the early prediction of impaired neurodevelopment in later life has been recently reported.[27,28] All brain volumes, particularly the cerebellar and thalamic volumes, have the best ability to predict normal neurodevelopment at 2 years.[27] A 3D-cUS volumetric assessment at postnatal days 30–40 has excellent accuracy, high intra- and extra-operator reproducibility, and is efficacious in terms of time, cost, and feasibility[27] (Table 1).

Magnetic Resonance Imaging

Magnetic resonance imaging has the highest resolution for detecting cerebellar abnormalities including hemorrhages (8–24% in term–equivalent age MRI in VP infants). Conventional T1- and T2-weighted sequences can identify most abnormalities at



Figs 1A and B: (A) Normal supra-tentorial structures (sagittal view) visualized by cUS in a preterm infant through AF; (B) View (sagittal view) showing cerebellum (marked in yellow)

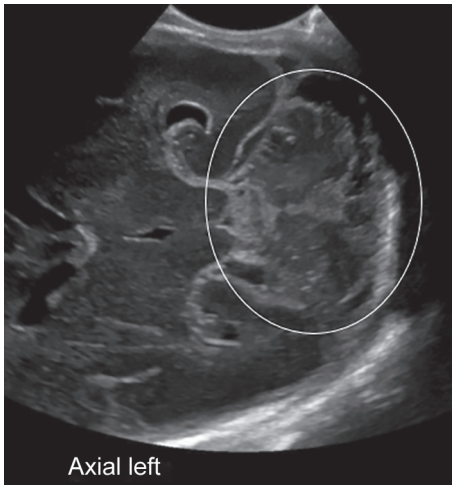


Fig. 2: Brainstem and cerebellum (encircled) visualized through left mastoid fontanelle (MF)

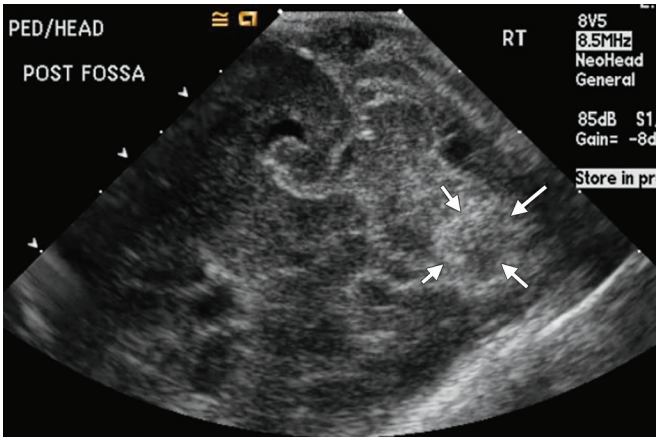


Fig. 3: Sonographic image of the cerebellum obtained through the right mastoid fontanelle in a premature infant on day of life 7, showing an ill-defined focal hyperechoic area in the left cerebellar hemisphere (white arrows) consistent with a grade 3 CBH

term–equivalent age, including CBH, hypoplasia, or acquired volume loss (Fig. 6). An MRI testing enables the detection of associated supratentorial injuries, and estimation of global brain development and maturation, hence, additional prognostic information. Susceptibility-weighted imaging can identify small punctate hemorrhages, which may be missed on conventional MRI.[30] An MRI can also help in the recognition of posterior fossa anomalies such as the Dandy–Walker malformations (Fig. 5B).

Volumetric MRI

A volumetric MRI is an advanced modality for MRI that can help assess cerebellar growth. The cerebellar volumes can be compared

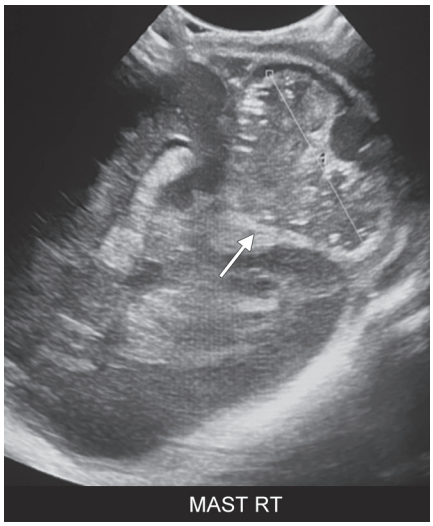
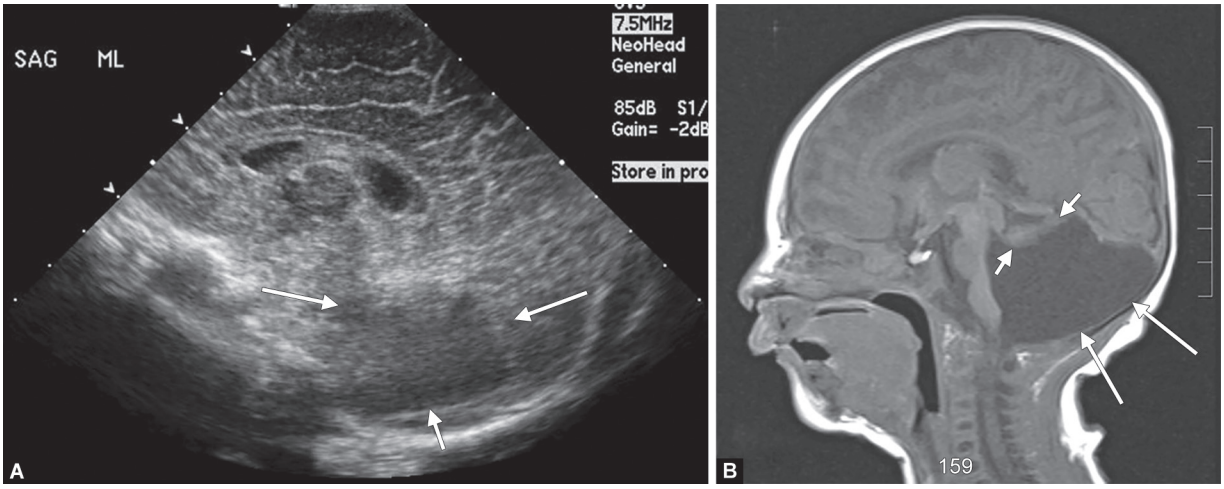


Fig. 4: Coronal USG image obtained on postnatal day 6 in this premature infant born at 26 weeks' gestation. The posterior fossa/cerebellum is seen through the right mastoid fontanelle (the image was obtained to measure the transverse cerebellar diameter at the site of maximum width of the cerebellar hemispheres [line])

with available reference ranges at term–equivalent age. In their systematic review and meta-analysis including 791 VP infants, Romberg et al. reported that the mean cerebellar volume at term–equivalent age was (mean ± standard deviation) 21 ± 6 mL.[31] They concluded that MR-based measurement of cerebellar volume could serve as a surrogate outcome for neurodevelopment.[31] Kim et al. compared cerebellar and other regional brain volumes in VP infants with isolated CBH. The volumetric analysis showed that the isolated CBH group showed smaller cerebellum (11.7 ± 3.0 vs 13.5 ± 3.0 mL, $p = 0.007$) and pons (1.5 ± 0.4 vs 1.8 ± 0.3 mL, $p = 0.001$) at term–equivalent age. In the subgroup analysis of infants with neurodevelopmental impairment, the ventral diencephalon and midbrain had significantly smaller volumes at 24-month corrected age.[32]

Volumetric MRI has also been used to create parametric surface models using the spherical harmonic description (SPHARM). Images from "control" infants and others with structural variations in the shape of the developing cerebellum have been compiled in an "atlas," which is a useful visual reference. Wu et al. reported altered global, regional, and local development of cerebellum in the absence of structural brain injury on MRI. They have described the longitudinal growth rates of various parts of the cerebellum and shape differences in 74 preterm infants without evidence of



Figs 5A and B: (A) Sagittal midline cUS image of the neonatal brain obtained *via* the AF, showing a small hyperechoic remnant of the cerebellum located superior to a cystic area occupying the entire enlarged posterior fossa (marked by arrows) consistent with Dandy–Walker malformation; (B) Sagittal midline T1-weighted MRI of the same infant—as in subpart (A)—a neonate showing a small remnant of the superior portion of the cerebellar vermis (small arrows) along with an enlarged cisterna magna (large arrows). The rest of the cerebellum is absent. Findings consistent with Dandy–Walker malformation

Table 1: Summary of the commonly used imaging modalities and sensitivity for diagnosing CBH[21,22,25,29]

Imaging modality	*Structures visualized*	*Sensitivity of imaging modality*
cUS: AF	Supratentorial structures and related lesions	17% for limited CBH
cUS: MF	Brainstem, cerebellar hemispheres, cerebellar vermis, cisterna magna, and fourth ventricle Measurement of TCD for cerebellar growth	83% for limited CBH 100% for large CBH
cUS: PF	Choroid plexus, cerebellar hemispheres, lateral ventricle trigone, occipital horn, occipital lobe, subarachnoid space, and tentorium	Data not available
MRI T1W	Supratentorial and infratentorial structures	80% for punctate CBH 100% for limited and large CBH

AF, anterior fontanelle; cUS, cranial ultrasound; MF, mastoid fontanelle; MRI T1W, T1-weighted magnetic resonance imaging; PF, posterior fontanelle; TCD, transcerebellar diameter

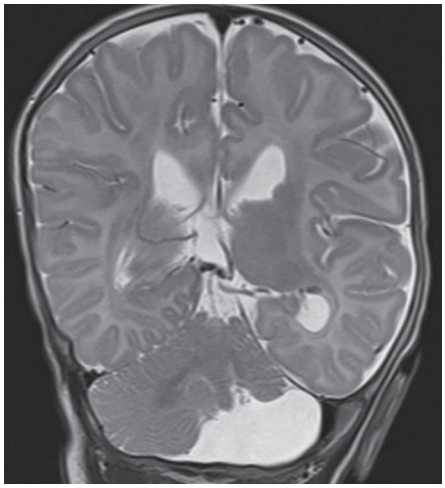


Fig. 6: A T2-weighted MRI showing posthemorrhagic cerebellar hypoplasia

structural brain injury and compared them with healthy *in utero* fetuses ($n = 38$) and term infants ($n = 38$). Premature infants had smaller cerebellar hemispheres and lower total cerebellar volumes compared to *in utero* healthy fetuses. The vermian region was significantly larger in preterm infants compared to control fetuses. At term–equivalent age, premature infants had smaller cerebellar hemispheres bilaterally, extending to the superior aspect of the left cerebellar hemisphere, and larger anterior vermis and posteroinferior cerebellar lobes compared to healthy term infants.[33] Smaller cerebellar volumes and slower cerebellar growth rates are associated with adverse neurodevelopmental outcomes.[34]

Diffusion-weighted MRI

Diffusion-weighted MRI is a technique used to assess white matter microstructural development. It measures Brownian motion of water in brain tissue and quantifies it as the apparent diffusion coefficients (ADCs).[35] The motion of water in the brain is not random; it is impeded by white matter or axons, and this directional dependence of the movement of water molecules is measured as "fractional anisotropy (FA)" values. Diffusion-weighted imaging (DWI) measures the random motion of water molecules in brain tissues and employs diffusion gradients to detect changes in water diffusion with high sensitivity. DWI is particularly useful in identifying acute or recent brain injuries or ischemic strokes, as areas of restricted diffusion appear as bright spots on DWI images. It provides information regarding the diffusion of water, but no information regarding its direction. In contrast, diffusion tensor imaging (DTI) measures both the magnitude and the direction of water diffusion in brain tissue. Multiple diffusion gradients determine the diffusion tensor (DT), a 3×3 array of numbers corresponding to diffusion rates in each combination of directions. The DT depicts each brain voxel's 3-dimensional diffusion properties. The axonal tracts connecting the different brain areas can be visualized using DTI and 3D reconstruction, referred to as "tractography." DTI is beneficial for studying brain connectivity, mapping white matter pathways, and investigating diseases such as preterm white matter damage, neurodegenerative diseases, and brain tumors. Diffusion-weighted MRI has been used to study microstructural alterations in the cerebellum. Brossard–Racine et al. demonstrated that FA is increased in the middle cerebellar peduncle and dentate nuclei in VP infants at term–equivalent age compared to term–born controls.[36] Although increased FA suggests maturation of the white matter tracts, other conditions such as reduced axonal thickness, reduced crossing of fibers and reduced dendritic branching also contribute to raised values. The neurodevelopmental implications of such microstructural differences in preterm infants need to be further defined. Cerebellar ADC values correlate with gross motor outcomes. Increased cerebellar ADCs at term–equivalent age were reflective of cerebellar underdevelopment, and lower size and cell density in the molecular and internal granular layers.[37]

Proton MR Spectroscopy

Proton MR Spectroscopy of the cerebellum at term–equivalent age is being evaluated as a tool to predict neurodevelopmental outcome in preterm infants. Proton MR spectroscopy measures various brain metabolites, relative and absolute concentrations of which change rapidly with brain maturation. Moreover, N-acetylaspartate (NAA) is an amino acid synthesized primarily in neurons and/or axonal mitochondria and is a marker of neuronal activity.[38] Choline can be a marker for membrane turnover and myelination, and lactate reflects brain energy metabolism.[39] Also, NAA increased, whereas choline and lactate decreased with progressive brain maturation. Cerebellar NAA, choline, and creatine were positively correlated, whereas the choline/creatine ratio was negatively correlated with increasing postmenstrual age. The cerebellar NAA/choline ratio at term–equivalent age has been positively related with cognition but not motor outcomes at 2-year corrected age.[40] Brossard–Racine et al. explored the associations between altered cerebellar metabolite profiles and topography and severity of brain injury in 59 VP infants compared to 61 term controls. Furthermore, VP infants had lower cerebellar NAA ($p < 0.025$) and higher choline ($p < 0.001$) at term–equivalent age than healthy term controls. Cerebellar injury was consistently associated with reduced NAA, choline, and creatine values. They concluded that infection, cerebellar injury and supratentorial injury were important risk factors for impaired preterm cerebellar biochemistry.[41]

Functional MRI

Functional MRI uses blood oxygenation level-dependent (BOLD) sequences to measure the local tissue-level imbalance between oxygenated and deoxygenated hemoglobin. This generates a contrast which is seen as an image. Typically, in adult or older children population, functional MRIs are task-based and need the cooperation of the patient to exactly visualize the parts of the brain which are active during the task. However, there are synchronized low-frequency fluctuations of BOLD signal which are observed even during resting state in brain tissues, and this has been described as the resting-state functional MRI (rs-fMRI). The neuronal networks identified are referred to as "resting-state networks" (RSN).[42] Herzmann et al. described the rs-fMRI findings in VP infants and compared the intracerebellar and cortico–cerebellar connections between VP infants without major intracranial pathology on earlier USG/MRI at term–equivalent age, with term controls.[43] Cortico–cerebellar functional connectivity was well-established by term age. VP infants had reduced magnitudes of cortico–cerebellar correlation, but no alterations in intra- and cortico–cerebellar functional connectivity topography.

An rs-fMRI has also been used to study the cerebello–cortical connections in behavioral and ASDs. The cerebello–cortical connections to the somatosensory and motor cortex are overdeveloped and the connections to the association areas are

underdeveloped in ASDs.[44] Uusitalo et al. have reported brain activation in fMRIs of VP infants compared to term-born controls at 13 years of age. They found stronger activation in the right cerebellar lobule V and left cerebellar lobule VI during finger opposition and stronger activation in the right superior parietal lobule during dyadochokinesis in left-hand tasks.[45]

Computed Tomography (CT)

Cerebellar abnormalities can be visualized by CT but it has no added diagnostic value compared to cUS and MRI.[46] Furthermore, CT scan involves exposure to ionizing radiation. The CT is hence recommended in potential neurosurgical emergencies such as acute symptomatic posterior fossa hemorrhage, where cUS is inconclusive and MRI is not readily available.[47]

Brain Function Assessment Using Combined Neuroimaging and Electroencephalopgraphy (EEG)

In their prospective study of 64 VP infants, van 't Westende et al. investigated associations between motor outcome and brain volumes (white matter, deep gray matter, cerebellum, and ventricles), white matter integrity (FA; mean; and axial and radial diffusivity) and brain activity (upper alpha/A2 functional connectivity and relative A2 power). Ventricular volume and relative A2 power were independently associated with motor outcome at 9–11 years.[48] De Wel et al. showed that the continuity and complexity of EEG steadily increased with increasing postnatal age and influenced cerebellar size. They concluded that excitatory neuronal activity stimulated myelination and thus increased brain activity affected structural development.[49]

Author Contributions

PK, SD, and JLA wrote first and current draft under supervision of GJ; GJ conceptualized and designed the project, supervised first draft, and significantly revised to current draft version of manuscript; NP, RH, AU, and AR contributed to final draft and provided images for completion of the manuscript; and TH provided expert intellectual input into the manuscript and revision to current draft.

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ORIGINAL RESEARCH

Evaluation of a Cranial Ultrasound Scoring System for Prediction of Abnormal Early Neurodevelopment in Preterm Infants

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Abstract

Aim: To evaluate and compare a cranial ultrasound (cUS) scoring system to conventional reporting of cranial ultrasound abnormalities (CUAs) for prediction of early neurodevelopmental outcomes in preterm infants.

Materials and methods: This retrospective, single-center study compared cUS scores to results from late ultrasound examination reports for any cUS abnormality (CUA) (any hemorrhage or white matter lesion) or severe CUA [severe intraventricular hemorrhage (IVH)], cystic periventricular leukomalacia (PVL), parenchymal or cerebellar hemorrhage) for predicting early signs of cerebral palsy (CP) or developmental delay in preterm infants.

Results: Six-weeks postnatal cUS examinations were analyzed against early neurodevelopmental outcomes at 3–4-months corrected age of 242 preterm infants (median gestational age, 26.5 weeks; interquartile range [IQR, 4 weeks] and median body weight 880 grams [IQR, 356.5 grams]). We did not find any statistically significant differences between cUS score and any CUA for sensitivity (57% vs 57% [95% confidence interval (CI): from −19 to 19]) and specificity (68% vs 64% [95% CI: from −3 to 10]) for predicting CP. Similarly, there was no difference in sensitivity (44% vs 46% [95% CI: from −12 to 7]) and specificity (74% vs 70% [95% CI: from −5 to 13]) for predicting any developmental delay. However, in comparison to severe CUA, cUS score had significantly higher sensitivity (57% vs 27% [95% CI: from 12 to 49]) but significantly lower specificity (68% vs 96% [95% CI: from −21 to −34]) for predicting CP. There was higher sensitivity (44% vs 12 % [95% CI: from 23 to 41]) but lower specificity (74% vs 98% [95% CI: from −15 to −32]) for any delay.

Conclusions: Cranial ultrasound score was similar to any reported CUA for predicting neurodevelopmental outcomes; however, when compared to severe CUA, it had better sensitivity but poor specificity for predicting early neurodevelopmental outcomes.

Clinical significance: Objective scoring of cUS examinations on late neonatal scans was found to be similar to conventional reporting of any CUA for the prediction of early neurodevelopmental outcomes in this retrospective study. This indicates that scoring does not value add to the diagnosis of these infants.

Keywords: Brain injury, Cerebral palsy, Cranial ultrasound, Early intervention, Neurodevelopmental outcome, Preterm infants, Prognosis.

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Introduction

Cranial ultrasound (cUS) is widely used for the screening of preterm neonates to detect brain injury due to prematurity.[1,2] Infants found to have brain abnormalities on cUS are followed up with further imaging,[3] focused neurodevelopment assessments,[4] and early intervention provided when indicated.[5,6] Brain injuries that are predictive of early neurodevelopmental impairment in preterm infants include any grade of intraventricular hemorrhage (IVH), cerebral or cerebellar hemorrhage, white matter injury (WMI), ventriculomegaly and hydrocephalus, cystic changes, and signs of brain atrophy after any injury.[7,8]

Late screening cUS examinations are performed at various time points in different institutions;[9] 6-weeks, term–equivalent age (TEA) or discharge may be used as the last screening examinations. The late cUS examination is important to detect any evidence of WMI.

The use of a cUS scoring system (Appendix 1) for the quantification of brain injury in preterm neonates has been reported as a useful tool to consider using in the prediction of neurological outcome.[10,11] The scoring system includes measurements of the lateral ventricle, interhemispheric fissure, thickness of the corpus callosum and subarachnoid space. Several subjective assessments of injury are also included in the score items indicating signs of white matter loss, the presence of any cysts and deep gray matter injury. These assessments

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were obtained from a TEA cUS performed on the neonates. The cUS score provides a systematic approach to quantifying brain abnormality in a single score and the higher the score the more likely the expected outcome of adverse neurodevelopment. However, the data was limited on the validity of this technique as a reliable method of predicting neurological outcome and we, therefore, studied a group of high-risk neonates to assess its usefulness in predicting adverse early neurodevelopmental outcomes at 3–4-months corrected age (age from the original due date).

Early neurodevelopment assessment provides screening for early features of cerebral palsy (CP) or early developmental delay in infants who are born preterm and require intervention.[12]

The aim of this study was to evaluate and compare the parameters of two methods (cUS scoring vs conventional cUS reporting) to characterize abnormal cUS findings for early adverse neurodevelopmental outcomes—any CP or any adverse neurodevelopment assessed at 3–4-months corrected age.

Materials and Methods

The cUS screening is routinely performed on preterm infants, who are born in less than 32 weeks gestational age (GA) or below 1500 gm in our unit. The screening includes a *late* or last cUS examination when neonates are 6-weeks postnatal age (PNA). Our unit does not perform a TEA cUS as part of the screening protocol. Infants born before 29 weeks GA or with a birth weight of less than 1000 gm were selected for inclusion in this retrospective cohort study as they were the group who had routine inpatient and outpatient neurodevelopmental assessments in our unit. The data available for the study was obtained from the time of the establishment of the neurodevelopment clinic in 2018 until 2022. This includes screening for early features of CP or developmental delay at 3–4-months corrected age.[4,12] Neonates were excluded from the study if they did not have a 6-weeks PNA cUS examination prior to their early neurodevelopment clinic assessment. Ultrasound examinations were performed to routine screening standards of our unit at the time and neurological examinations performed on each neonate in the study in a dedicated early neurodevelopment clinic.[12,13]

The cUS score was calculated retrospectively by two investigators (GM and KVH) from the ultrasound examination reports using the cUS scoring system reported previously,[10,11] and both scorers were blinded to the early neurodevelopment clinic outcomes. In this study, a cUS score of 10 or lower was considered normal or test negative, whereas a score of more than or equal to 11 was considered abnormal or test positive.[10] If the scores were different and one scorer considered the cUS examination was normal, while the other scorer scored as abnormal (10 vs ≥11) a consensus was reached after deliberation. Any cUS abnormality was considered positive if there was the presence of any abnormality, such as IVH, periventricular leukomalacia (PVL), cerebral or cerebellar hemorrhage and negative if the cUS examinations demonstrated no abnormality,[8] Similarly, the severe CUA test was considered positive if there was IVH grade 3 or above present,[14,15] cystic PVL,[16] cerebral (parenchymal) or cerebellar hemorrhage. Otherwise, the test outcome was considered negative.

The cUS examinations included static coronal and sagittal images obtained through the anterior fontanelle and axial plane images were obtained through the mastoid fontanelle.[1,17,18] All examinations were performed by in-house trained and credentialled sonographers and the examinations were reported by consultant pediatric radiologists. No extra projections were required to calculate the cUS scores and it was, therefore, possible to use available images and imaging reports.

The neurodevelopment assessments included a video assessment of general movements [general movements assessment (GMA) at fidgety age],[19] Hammersmith Infant Neurological Examination (HINE),[20] and medical examination for the outcomes of early features of CP, high risk of CP, developmental delay, abnormal GMA, and suboptimal HINE.

Statistical Analysis

Statistical analyses were performed using STATA, version 17.0 (StataCorp LLC, College Station, Texas, USA). The sensitivities and specificities were calculated for the index diagnostic tests (cUS score, any CUA, and severe CUA) against the reference outcomes of an infant with CP (clinic diagnosis of early features of CP or high risk of CP), and a composite of any developmental delay (any abnormality in development, early features of CP, or high risk of CP). Absolute differences in the test accuracy with 95% confidence interval (CI) were calculated to compare the differences between the sensitivities and specificities of the index diagnostic tests accuracy using the McNemar's test. Although cUS score above 10 was considered as abnormal based on the previous literature, the receiver operating characteristic (ROC) analysis was also performed to determine the cut-off value for cUS score that most accurately predicts CP and for any developmental delay.

Results

Two hundred and forty-two preterm infants were included in the study with the median GA being 26.5 weeks (IQR, 4 weeks). The median body weight was 880 grams (IQR, 356.5 gm). Any CUA was reported in 93/242 (38%) infants and 17/93 (18%) had severe CUAs. Furthermore, 85/242 (35%) infants had an abnormal score of more than or equal to 11. The median score was similar between the two scorers (medial 10 vs 10) and there was no disagreement ($\kappa = 1$). At the early neurodevelopment clinic assessments, there were 33/242 (14%) infants diagnosed with CP/high risk of CP and 124/242 (51%) had some form of developmental delay.

Cranial Ultrasound Score vs Any Cranial Ultrasound Abnormalities

There was no difference in sensitivity (57% vs 57% [95% CI: from −19 to 19]) and specificity (68% vs 64% [95% CI: from −3 to 10]) for predicting CP. There was no difference in sensitivity (44% vs 46% [95% CI: from −12 to 7]) and specificity (74% vs 70% [95% CI: −5 to 13]) for predicting any delay (Table 1).

Cranial Ultrasound Score vs Severe Cranial Ultrasound Abnormalities

There was significantly higher sensitivity (57% vs 27% [95% CI: from 12 to 49]) but lower specificity (68% vs 96% [95% CI: from −21 to −34]) for predicting CP. There was higher sensitivity (44% vs 12% [95% CI: from 23 to 41]) but lower specificity (74% vs 98% [95% CI: from −15 to −32]) for any delay (Table 2).

The ROC curve (Fig. 1) demonstrated that a cUS score of above or equal to 10.5 had a sensitivity of 52% for predicting CP with a specificity of 19% (Area under the curve (AUC): 0.66; 95% CI: from 0.55 to 0.77).

The ROC curve (Fig. 2) demonstrated that a cUS score of above or equal to 10.5 had a sensitivity of 23% for predicting any adverse

Table 1: Diagnostic test accuracy of abnormal cUS score vs any CUA for predicting adverse early neurodevelopmental outcomes

	cUS score	*Any CUA*	*Absolute difference in the test accuracy (95% CI) (%)*	*p-value*
Outcome: CP/ high risk of CP				
Sensitivity (%) (TP/TP + FN)	57 (19/33)	57 (19/33)	0 (−19, 19)	1.00
Specificity (%) (TN/TN + FP)	68 (143/209)	64 (135/209)	4 (−3, 10)	0.25
Outcome: Any delay or CP/ high risk of CP				
Sensitivity (%) (TP/TP + FN)	44 (55/124)	46 (58/124)	−2 (−12, 7)	0.60
Specificity (%) (TN/TN + FP)	74 (88/118)	70 (83/118)	4 (−5, 13)	0.31

CP = cerebral palsy; CUA = cranial ultrasound abnormality reported on cranial ultrasound examination; any delay = any neurodevelopmental delay at 3–4 months corrected age

Table 2: Diagnostic test accuracy of abnormal cUS score vs severe CUA for predicting adverse early neurodevelopmental outcomes

	cUS score	*Severe CUA*	*Absolute difference in the test accuracy (95% CI) (%)*	*p-value*
Outcome: CP/ high risk of CP				
Sensitivity (%) (TP/TP + FN)	57 (19/33)	27 (9/33)	30 (12, 49)	0.002
Specificity (%) (TN/TN + FP)	68 (143/209)	96 (201/209)	−28 (−21, −34)	0.0000
Outcome: Any delay or CP/high risk of CP				
Sensitivity (%) (TP/TP + FN)	44 (55/124)	12 (15/124)	32 (23, 41)	0.0000
Specificity (%) (TN/TN + FP)	74 (88/118)	98 (116/118)	−24 (−15, −32)	0.0000

CP = cerebral palsy; severe cranial ultrasound abnormality = severe intraventricular hemorrhage (IVH), cystic periventricular leukomalacia (PVL), parenchymal or cerebellar hemorrhage; any delay = any neurodevelopmental delay at 3–4 months corrected age

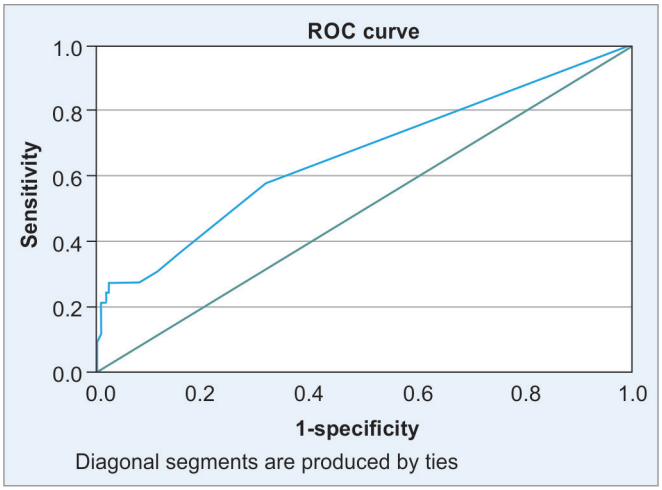

Fig. 1: An ROC curve demonstrating that cUS score of above or equal to 10.5 had a sensitivity of 57% for predicting CP with a specificity of 31% (AUC: 0.66; 95% CI: from 0.55 to 0.77)

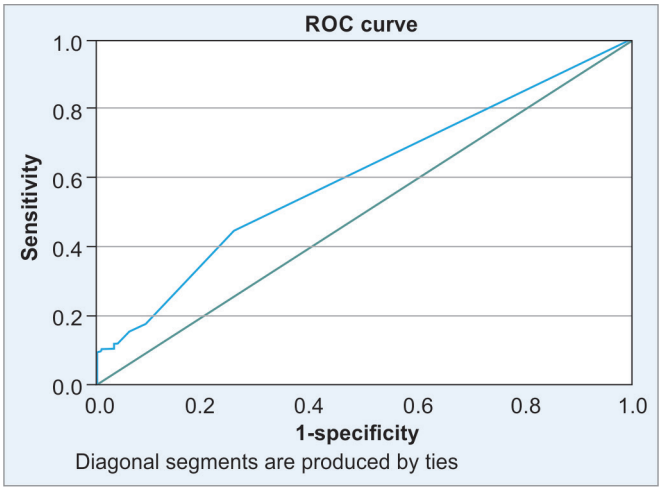

Fig. 2: An ROC curve demonstrating that cUS score of above or equal to 10.5 had a sensitivity of 44% for predicting any adverse neurodevelopment delay with a specificity of 25% (AUC: 0.6; 95% CI: from 0.53 to 0.67)

neurodevelopment delay with a specificity of 25% (AUC: 0.60; 95% CI: 0.53 to 0.67).

Discussions

We report the practical use of a cUS scoring system[10] to predict early neurodevelopmental outcomes in a cohort of extremely preterm neonates. The cUS score in our study had no difference in sensitivity for the prediction of CP but lower sensitivity for any neurodevelopment delay, as compared to CUA. However, the specificity of using the cUS score was higher for both CP and any delay. Compared to severe CUA, the score had significantly higher sensitivity for predicting CP and any delay but had significantly lower specificity for CP and any delay. Overall, the cUS score performed the same when compared to any CUA but had higher sensitivity and lower specificity when compared to severe CUA when predicting abnormal early neurodevelopment in extremely preterm infants.

The literature reporting the use of cUS scoring is sparse and our study adds to the evidence on this emerging technique. The scoring system is the only cUS scoring system documented. The study by Skiöld et al. carried out on 84 infants found that

agreement between the cUS scoring system and MRI scores was good. Sensitivity was the same for cUS and MRI in predicting CP (75%) and severe cognitive delay (100%). Specificity for CP was the same for MRI compared with cUS (97% vs 90%).[10] Our study in comparison had lower sensitivity than theirs for predicting CP (57% vs 75%). The specificity to predict CP in our study was lower than Skiöld et al. (68% vs 90%), although the ultrasound examinations were performed at different time points with our infants examined at 6-weeks PNA while the Skiöld et al. infants were all TEA when the cUS score was calculated. The sample size of the Skiöld was however smaller than our study (84 vs 242).

This cUS score has been reported as having similar sensitivity to MRI brain in a study on infants at TEA.[10] The score includes an expanded list of brain injuries compared with other studies[21,22] and is therefore more in line with MRI scoring systems.[23–25] This includes subtle abnormalities like corpus callosum thinning and delayed folding of the cortex as well as the sequelae of IVH, periventricular hemorrhagic infarction (PVHI), ventriculomegaly and injury to the white matter including any signs of atrophy. Although their study used the cUS score to compare cUS findings at TEA with MRI, it is reasonable to suggest that it could also be of value when comparing cUS examinations. Unlike the MRI and cUS studies on infants at TEA, our study evaluated data from ultrasound examinations performed at 6-weeks PNA, as this is the final screening examination performed at our unit. We acknowledge that further white matter volume loss could have occurred in the infants after the 6-weeks cUS examination and therefore not captured by the study. Further studies to assess later cUS score at TEA should be therefore investigated in a similar group of infants.

A strength of our study was the 100% agreement between the two scorers when deciding if a score was normal or abnormal (abnormal ≥ 11) and our study had a considerable larger cohort compared to the previous Skiöld study. Although our study has a relatively big sample size, compared with the Skiöld study, some CI are very wide and the study would benefit from a larger patient group. As the cUS score was possible to be calculated at a later date than when the examination was performed, it could be calculated by an independent operator in an auditing or research setting.

Another limitation of the study was assessing the cUS score in a dichotomous way to calculate the diagnostic accuracy parameters. The cUS score is a continuous measure with a score of 10 being normal and any score above 10 abnormal—the higher the score the more likely there is brain injury. However, we have used it as a categorical variable as a distinction between normal and abnormal is required to calculate the sensitivity and specificity. Therefore, we also performed an ROC analysis, which shows that the cut-off used in the study agrees with the previous study.[10] The AUC of the ROC curve of 0.5 indicates that the performance of the tests are not significantly different for predicting early abnormal neurodevelopment and is of very limited value.

The late cUS examinations at our institution were performed at 6-weeks PNA, according to our protocol, rather than at TEA as described by Skiöld et al. This meant that the age of the neonates in our study was varied unlike those in the published study. It may indicate that this scoring system is only predictive when used for term-aged neonates. We plan to conduct future studies, which look at late screening time points to evaluate this further.

All infant's neurodevelopment was assessed early at 3–4-months corrected age. The infants will also be undergoing later neurodevelopment assessments at two years of age. Early diagnosis allows for early intervention and treatment,[5,13] and any adverse neurodevelopment would be confirmed with longer term follow-up. As the study shows the overall cUS scores are quite low and therefore ultrasound poor at predicting abnormal neurodevelopment it is crucial there is follow-up in all early preterm infants. Severe CUA has good specificity but very poor sensitivity and therefore it is important it is not considered as sufficient to use alone.

Conclusions

The use of a cUS scoring system to predict early neurodevelopmental outcome is similar to any reported CUA for predicting outcomes, however, when compared to severe CUA, it had better sensitivity but poor specificity for predicting abnormal early neurodevelopmental in extremely preterm neonates.

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Appendix 1: Cranial ultrasound scoring system.[1]

		Subjective assessment				
Scoring item		*Score 1*	*Score 2*	*Score 3*	*Score 5*	*Score 10*
I	Cysts or cavity	None		Focal cyst or cavity but not involving cortico-spinal tract	Unilateral cyst or cavity involving more than one region but not cortico-spinal tract or optic radiation	Cyst or cavity involving cortico-spinal tract or bilateral cystic PVL
II	Cortical gray matter abnormality	None	One focal abnormality		Extensive abnormality	
III	Deep gray matter abnormality	None			Unilateral atrophy/cysts	Bilateral atrophy/cysts
IV	Maturation of gyral fold	Normal	Frontal reduction of gyral folding	Global reduction of complex gyral folding/ delayed gyration for gestational age		
V	Cerebellar abnormality	None	Small focal hemorrhage	Unilateral extensive lobar hemorrhage	Bilateral extensive lobar hemorrhage	
Measured items						
VI	Size of frontal horns Ventricular index Anterior horn width	Normal <13 mm <3 mm	Moderate dilatation 13–16 mm 3–6 mm		Severe dilatation or shunt without dilatation >16 mm >6 mm	Shunt with persistent dilatation
VII	Size of ventricular midbody	Normal <10 mm	Mild-moderate enlargement 10–15 mm	Severely enlarged >15 mm		
VIII	Subarachnoid space size	Normal <4 mm	Mildly enlarged 4–6 mm	Severely enlarged >6 mm		
IX	Size of inter-hemispheric fissure	Normal <3 mm	Mildly enlarged 3–6 mm	Severely enlarged >6 mm		
X	Thickness of Corpus callosum	Normal >1.5 mm		Marked thinning <1.5 mm		

ORIGINAL RESEARCH

Enteral Feeding and Antibiotic Treatment Do Not Influence Increased Coefficient of Variation of Total Fecal Bile Acids in Necrotizing Enterocolitis

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Abstract

Introduction: Necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency in preterm infants. In animal models, the accumulation of ileal bile acids (BAs) is a crucial component of NEC pathophysiology. Recently, we showed that the coefficient of variation of total fecal BAs (CV-TBA) was elevated in infants who develop NEC compared to matched controls. However, neither the type of enteral nutrition nor antibiotic treatments—parameters that could potentially influence BA levels—were used to match pairs. Thus, we assessed the relationships between exposure to enteral feeding types and antibiotic treatments with NEC status and CV-TBA.

Materials and methods: Serial fecal samples were collected from 79 infants born with birth weight (BW) ≤1800 gm and estimated gestational age (EGA) ≤32 weeks; eighteen of these infants developed NEC. Total fecal BA levels (TBA) were determined using a commercially available enzyme cycling kit. Relationships between CV-TBA and dichotomous variables (NEC status, demographics, early exposure variables) were assessed by independent samples t-tests. Fisher's exact tests were used to assess relationships between NEC status and categorical variables.

Results: High values for CV-TBA levels perfectly predicted NEC status among infants in this study. However, feeding type and antibiotic usage did not drive this relationship.

Conclusions: As in previous studies, high values for the CV-TBA levels in the first weeks of life perfectly predicted NEC status among infants. Importantly, feeding type and antibiotic usage—previously identified risk factors for NEC—did not drive this relationship.

Keywords: Antibiotics, Bile acids, Baby, Enteral nutrition, Infant, Necrotizing enterocolitis, Newborn, Neonate.

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Introduction

Worldwide, necrotizing enterocolitis (NEC) is the most common gastrointestinal emergency of preterm infants with a birth weight (BW) of below 1500 gm.[1,2] Characterized by an inflammatory, hemorrhagic necrosis of the distal ileum and colon,[3] the clinical presentation of NEC ranges from abdominal distension to intestinal gangrene and bowel perforation.[4] In the United States alone, thousands of pre-term infants develop NEC with mortality rates ranging 20–40%.[1,5–7] Disease-associated costs are significant: preterm infants diagnosed with NEC remain hospitalized for an average of 43 days[8] with yearly costs estimated in billions of US dollars.[9] Patients with necrotic bowel often go on to develop short bowel syndrome, which is also associated with significant complications and prolonged medical expenses. In addition, surgical intervention in NEC is a strong predictor of neurodevelopmental morbidity.[10] The pathophysiology of this disease remains poorly understood, and non-surgical treatment strategies are mainly supportive. Currently, no predictive tests are approved to identify which infants will develop NEC, and by the time NEC is diagnosed clinically, intestinal damage has already occurred.

Bile acids (BAs) are required for emulsification, absorption, and transport of fats, sterols, and fat-soluble vitamins in the intestine and liver. Furthermore, BA homeostasis is a complex process involving coordinated synthesis from cholesterol in the liver, transport from the liver to the intestine, followed by transport back to the liver. If enterohepatic circulation is interrupted, accumulation of cytotoxic BAs can result in damage to the intestinal epithelium.[11,12] Also, BA-induced cellular disruption—largely a result of their detergent-like properties—can cause further damage through the release of inflammatory mediators. We have previously shown that the accumulation of ileal BAs is crucial to NEC pathophysiology.[13–15] Our most recent publication—using nine matched subject pairs, each with five paired samples based on the day of life when the samples were collected—showed a statistically significant increase in the coefficient of variation of total fecal BAs (CV-TBA) in infants who develop NEC compared to matched controls. Notably, there was a perfect prediction of NEC, and the increases in CV-TBA occurred well prior to clinical NEC diagnosis.[16]

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Compared to premature infants who are breastfed, formula-fed preemies are 6–10 times more likely to develop NEC[17] and have higher fecal BA levels.[18] Formula feeding is also required to develop experimental NEC.[19,20] In addition, while no specific pathogen has been conclusively associated with NEC,[21–29] the disease cannot be developed in germ-free conditions,[30,31] and colonization with specific species of gut bacteria is also required for formation of more cytotoxic secondary BAs.[32–34] Given that neither enteral nutrition type nor antibiotic treatments were used to match pairs in our previous publication,[16] and that these parameters could influence BA levels, it is possible they could also affect CV-TBA. Therefore, using a larger, unmatched cohort and without a standardized window for sample collection, we assessed relationships between exposure to enteral feeding types and antibiotic treatments, NEC status, and CV-TBA.

Materials and Methods

Study Participants

Following approval by the University of Arizona Institutional Review Board, premature infants were enrolled prospectively *via* informed, written parental consent at Banner University Medical Center Tucson. All research was performed in accordance with relevant regulations. The inclusion criteria—BW less than or equal to 1800 gm, estimated gestational age (EGA) less than or equal to 32 weeks, and below 30-days old prior to initiation of enteral feeding—were chosen because NEC occurs almost exclusively in premature infants, the most premature infants are more likely to develop the disease, and most cases occur after the initiation of enteral feeding.[4,35,36] Exclusion criteria included conditions not related to prematurity, including blood–culture positive sepsis or genetic syndromes and were based on eliminating subjects that could develop NEC-like syndromes due to other confounding problems not related to the most common risk factors for NEC. Definitions of NEC diagnosis and time of diagnosis were defined as any subject with Bell's Stage above or equal to II (modified Bell's staging criteria) and radiographic evidence of NEC, respectively. Feeding and antibiotic exposures were defined as a subject being given of any formula of any brand or type, donor or maternal breast milk, breast milk fortifier, any antibiotics, or specific antibiotics during the range of samples used for BA analysis.

Sample Collection and Analysis

Post-meconium fecal samples were collected from the diaper for up to four weeks after initiation of enteral feeding. Samples were placed in sterile microtubes, frozen in the NICU at –20°C and transported to the laboratory weekly where they were stored until processing. For analysis, samples were thawed, weighed, and mixed with an equal volume of nanopure water. After homogenization, samples were centrifuged to separate fecal water from the solids and the fecal water was frozen until BAs were assayed.[18,37] The Diazyme Total Bile Acids Assay Kit (Diazyme Laboratories, Poway, California, USA) was utilized to measure all BAs *via* an enzymatic cycling method with spectrophotometric readout.[13,14]

Statistics

For each infant, TBA levels across all stool samples were summarized in terms CV-TBA, calculated for each infant by dividing SD-TBA by mean-TBA. Relationships between NEC status and categorical variables (demographic and exposures) were described in terms of counts and percentages and assessed using Fisher's exact tests.

Table 1: Characteristics of cohort and samples analyzed

	Control (n = 61)	*NEC (n = 18)*	
	Mean ± SD	*Mean ± SD*	*p-value*
EGA (weeks)	27.6 ± 2.6	27.4 ± 2.6	0.7[1]
BW (gm)	1058 ± 303	968 ± 308	0.3[1]
% Male	57 (*n* = 35)	44 (*n* = 8)	0.4[2]
Sample #	19.8 ± 3.2	20.0 ± 4.4	0.9[1]
Sample DOL start	8.3 ± 3.5	7.8 ± 3.1	0.5[1]
Sample DOL end	29.3 ± 4.3	28.6 ± 3.8	0.5[1]

[1]*t*-test, unequal variances assumed. [2]Fisher's exact test. BW, birth weight; DOL, day of life; EGA, estimated gestational age

Table 2: Feeding practices and antibiotic use by NEC status

	Control (n = 61)	*NEC (n = 18)*	
	% (n)	*% (n)*	*p-value**
Feeding practice			
Formula	39.3 (24)	27.8 (5)	0.4
BM	95.1 (58)	88.9 (16)	0.3
Formula + BM	34.4 (21)	22.2 (4)	0.4
BM fortifier	95.1 (58)	94.4 (17)	1.0
Antibiotics			
Any antibiotics	24.6 (15)	33.3 (6)	0.5
Gentamycin	24.6 (15)	33.3 (6)	0.5
Ampicillin	23.0 (14)	33.3 (6)	0.4
Vancomycin	3.3 (2)	5.6 (1)	0.5
Other	1.6 (1)	5.6 (1)	0.4

*Fisher's exact test

Relationships between NEC status and continuous variables (CV-TBA, EGA, BW, sample number, and sample DOL start and end) were assessed by independent samples *t*-tests assuming unequal variances, as were relationships between CV-TBA and other dichotomous variables (demographics, early exposure variables).

Results

Among the 79 infants included in this study, 18 developed NEC within the first 39 days of life and the other 61 infants were selected as controls. Observation periods, EGA and BW were similar for control infants and those with NEC, as control infants were selected based on similar EGA and BW ranges to their NEC counterparts and were followed for similar times as NEC infants (Table 1). Comparisons of exposure prevalence between infants with NEC and unmatched controls for types of enteral feeding and antibiotic treatment during the range of samples used for analysis are shown in Table 2. No infants were exclusively formula fed, and in both groups, most patients received BM (maternal and/or donor), with a much smaller percentage receiving formula and BM as formula is given only when there is no consent for donor breast milk and maternal milk is not available. For this dataset, none of these factors showed a relationship to NEC.

Figure 1 shows the distributions of CV-TBA between groups. Notably, similar to what was shown previously using matched pairs,[16] CV-TBA has no overlap: all infants who developed NEC had CV-TBA greater than 0.8, and all infants who did not develop NEC, had CV-TBA less than 0.8.

Table 3 shows CV-TBA means and standard deviations (SDs) among all patients receiving (YES) or not receiving (NO) exposures to formula, breast milk (BM), breast milk fortifier (BM fortifier), formula and BM, any antibiotics, or specific antibiotics. Among these comparisons, there were no statistically significant relationships to CV-TBA. Figure 2 illustrates what is shown descriptively in the table: that the overall distribution and range of CV-TBA was similar for control and NEC infants fed formula (Fig. 2A) or given any antibiotics (Fig. 2B) compared to those who were not exposed to formula and antibiotics (Figs 2A and B, respectively). These points taken together demonstrate that the relationship between NEC and CV-TBA was independent of the relationship between CV-TBA and formula feeding or antibiotic treatment in this sample.

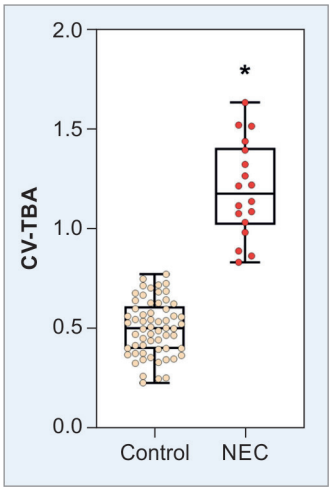


Fig. 1: CV-TBA by NEC status. Each point represents an individual subject's CV-TBA. *$p < 0.0001$

Table 3: CV-TBA[1] among all patients by feeding type and antibiotic exposure

Exposure (n)	*Yes*	*No*	*p-value*
Formula (29)	0.58 (0.32)	0.72 (0.33)	0.08
BM (75)	0.66 (0.33)	0.70 (0.36)	0.80
Formula + BM (54)	0.70 (0.33)	0.59 (0.34)	0.15
BM fortifier (75)	0.67 (0.34)	0.56 (0.20)	0.30
Any antibiotics (21)	0.66 (0.40)	0.67 (0.31)	0.90
Gentamycin (21)	0.66 (0.40)	0.67 (0.31)	0.90
Ampicillin (20)	0.67 (0.41)	0.66 (0.31)	0.90
Vancomycin (3)	0.72 (0.53)	0.66 (0.33)	0.90
Other (2)	1.11 (0.81)	0.66 (0.32)	0.60

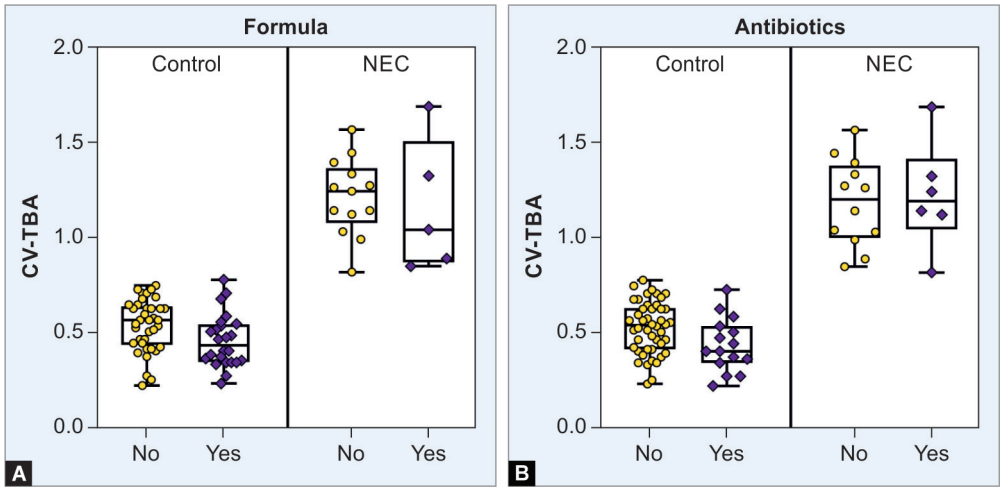
[1]Mean (SD).*t-test assuming unequal variances

Discussion

As previously shown[16] high values for the coefficient of variation (CV) of TBA levels perfectly predicted NEC status among infants in this study. Specifically, no control infants had CVs greater than 0.78, and no infants with NEC had CVs lower than 0.84, thus any threshold of detection set between 0.78 and 0.84 would have resulted in 100% sensitivity and 100% specificity in this sample. Importantly, feeding type and antibiotic usage—previously identified risk factors for NEC—did not drive this relationship.

By the time NEC is diagnosed clinically, intestinal damage has already occurred. An early marker is critical for reducing both morbidity and mortality. Current standard of care relies on monitoring preterm infants—particularly those with very low birth weight (VLBW; those born at less than 1500 g)—for clinical signs of NEC, such as feeding intolerance, vomiting, apnea, abdominal distension, or blood in stools.[38–40] The biomarkers that have been suggested for use in monitoring or diagnosing NEC—for example, C-reactive protein,[41] serum amyloid A,[42] calprotectin,[43] proinflammatory cytokines,[44–46] heart rate variability and peripheral oxygen saturation[47–49]—are similar to those found in sepsis, making differentiation between the two diagnoses problematic. Prediction of NEC by analyzing fecal microbiota,[50] proteomic,[51,52] or metabolomic[53,54] methods are more specific, but involve complex and expensive techniques that are not readily available in a clinical laboratory. Moreover, many of these proposed methods do not allow prediction with enough lead time for meaningful intervention. Increases of CV-TBA, however, occur well prior to NEC diagnosis.[16]

A common option for exploring the influence of formula feeding on the relationship between CV-TBA and NEC development



Figs 2A and B: Distribution of CV-TBA by (A) Formula feeding and NEC status and (B) Antibiotic treatment and NEC status. Each point represents an individual subject's CV-TBA

would be through the inclusion of formula feeding as a covariate with CV-TBA in a logistic regression model. However, given the complete separation in CV-TBA values by NEC status, a valid logistic regression model is not possible.[55] Visually, by displaying CV-TBA values by formula feeding and NEC status, we show the complete separation of CV-TBA values by NEC status, and that the overall distribution and range of CV-TBA was similar for infants fed formula compared to those who were not fed formula and infants given antibiotics versus those not given antibiotics. Similarly, the relationship between CV-TBA and development of NEC was also independent of whether the infant received both formula and breastmilk, BM fortifier, or specific antibiotic treatments (Table 3).

Other statistical characteristics of TBA levels (mean and SD) were strongly predictive of NEC (data not shown), but did not yield complete separation, i.e., there was overlap in the range of values between infants who developed NEC and control (data not shown). While it could be argued that because the CV is a function of the mean, specifically that the mean is in the denominator of the formula, that children with higher mean TBA levels would tend to have lower CVs, even if SDs were similar. This would not explain, however, our finding that high CVs were predictive of NEC. In fact, infants with NEC tend to have higher mean TBA levels, which would drive CVs downward rather than upward.

Because CV-TBA shows perfect prediction of NEC and is not influenced by enteral feeding or antibiotic treatment types, it is a promising candidate as a biomarker to predict development of this devastating disease. Further research is needed to assess these findings in a larger cohort and to fully develop and assess predictive models in order to initiate a multicenter trial to validate this biomarker.

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ORIGINAL RESEARCH

Linked Th17 and Calgranulin Responses in Maternal-cord Blood Dyads of Preterm Gestations with Histologic Chorioamnionitis

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Abstract

Introduction: Maternal–fetal immune crosstalk mechanisms are increasingly identified in the pathogenesis of gestational disorders, including histologic chorioamnionitis (HCA). Although an inflammatory Th17 immune phenotype has been described in preterm neonates with HCA, the associated maternal Th17 response is relatively unknown. To refine our understanding of Th17 biology in this context, we examined Th17 responses in maternal-cord blood dyads of preterm gestations.

Materials and methods: Paired maternal and cord blood (CB) samples were prospectively collected from preterm gestations (23–34 weeks) with HCA or controls. Th17-linked cell frequencies and plasma calgranulin (S100A8, S100A12) levels were determined by flow cytometry and enzyme-linked immunoassay, respectively.

Results: Analyses of 47 maternal-cord blood pairs showed striking parallel increases in Th17 cell frequencies as well as plasma calgranulin levels in the presence of fetal inflammation. Cord blood S100A12 levels were directly correlated with Th17 cell frequencies. In CB cultures, rh-S100A12 promoted *in vitro* propagation of Th17-type $CD4^+$ cells.

Conclusions: Maternal and CB Th17-linked responses are dually amplified in gestations with HCA, supporting a biological role for maternal–fetal interactions in this disorder. In addition to advancing current knowledge of neonatal Th17 mechanisms, these data shed new light on their association with maternal inflammation.

Keywords: Fetal inflammation, Gamma–delta T cells, Maternal inflammation, S100, S100A8, S100A12, Treg cells.

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Highlights

- The key message of our study is that certain parallel T-helper (Th) 17 cells and calgranulin responses can be found in mothers and cord blood (CB) of preterm gestations with histologic chorioamnionitis (HCA), particularly in the presence of fetal inflammation and despite the absence of maternal clinical symptoms.
- The effects of fetal inflammation on maternal and CB Th17 responses support mounting evidence of maternal–fetal inflammatory and immune crosstalk mechanisms.
- Calgranulins may be key mediators of perinatal inflammation modulated by the Th17 pathway.
- Our findings advance still limited understanding of the contributions of Th17 and calgranulin biology to placental, maternal, and CB inflammatory processes.
- This knowledge could be important to the targeted development of strategies to mitigate the pathogenesis of perinatal and neonatal inflammation.

Introduction

Preterm birth is a significant and increasing global health concern. The Centers for Disease Control reported that over 1 in 10 deliveries were preterm in the United States and that this number is increasing.[1,2] Of these, 70% were spontaneous, the result of preterm

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labor or preterm premature rupture of membranes (PPROM). Histologic chorioamnionitis (HCA) is a placental inflammation closely linked to spontaneous preterm birth.[3] Fetal exposure to HCA can result in adverse outcomes in preterm neonates, including brain injury, sepsis, necrotizing enterocolitis, and chronic lung disease, as detailed in a recent elegant review.[4] A postnatal diagnosis of HCA based on placental pathology is common in extremely preterm gestations, even in the absence of maternal or fetal symptoms associated with intra-amniotic infection (IAI).[5,6]

T-helper (Th) 17 cells represent a unique lymphocyte subset that can bridge adaptive and innate immune responses to protect the host against microbial pathogens.[7,8] Conversely, dysregulated Th17 cells may mediate pathologic processes leading to chronic inflammation of various organs, particularly affecting the brain in neonates.[9] Mounting evidence points to a role for Th17 cells in modulating immune function during normal pregnancy and in healthy neonates; however, much remains unknown, representing a significant knowledge gap.[10,11] In contrast, recent observations suggest potential contributions of Th17 cells to pathologic gestational processes including those leading to preterm delivery.[12–15] Also, Th17 cells may be functionally linked to members of the S100 family of proteins, including the calgranulins (S100A8, S100A9, S100A12).[16,17] While elevated calgranulin expression levels in association with HCA have been reported in placentas, amniotic fluid, and in preterm cord blood (CB) and neonatal blood,[18–21] a link between calgranulins and Th17 cells in the setting of HCA has not been established.

A growing body of literature suggests the importance of maternal–fetal immune crosstalk mechanisms to the pathogenesis of certain placental disorders, including chorioamnionitis.[22] However, whether systemic maternal Th17-type responses mimic the expression patterns found in their neonates, and whether such responses contribute to the pathogenesis of HCA, is presently unknown. Such observations could also be relevant to evidence that women who deliver a preterm infant are at increased risk of future heart disease,[23,24] particularly given the connection between enhanced Th17 responses and cardiovascular disorders.[25] We designed the present study to test the hypothesis that HCA is associated with enhanced circulating Th17 cell frequencies and Th17-linked calgranulin levels in affected pregnant women that parallel responses in the CB of their preterm neonates.

Materials and Methods

Human Subjects

From December 2016 to March 2019, eligible pregnant women admitted to the labor and delivery service at a large perinatal center in St. Louis, Missouri, USA were approached for their own enrollment and that of their delivered preterm neonates. Eligibility criteria included singleton or uncomplicated twin gestations, preterm labor and/or PPROM, and delivery between 23^0 and 34^6 gestational weeks. Potential subjects were excluded from study if mothers or pregnancies were affected by inflammatory conditions or infection other than suspected clinical chorioamnionitis, or if a potential for altered immunity related to congenital or genetic conditions in the fetus or newborn existed. Demographic and clinical details were obtained from the electronic medical record. This prospective observational study was performed with the approval of a protocol and according to the policies of the Institutional Review Board for Human Studies of Saint Louis University, SSM Health Cardinal Glennon Children's Hospital (CGCH), and SSM Health St. Mary's Health Center (SMHC). Informed, written consent was obtained for all study participants.

Diagnosis of Histologic Chorioamnionitis

All placentas were examined by a clinical academic pathologist as part of routine clinical care (Redline criteria[3]). Diagnosis and staging of HCA were based on the involved compartment (maternal and fetal) and the extent of neutrophil invasion.[3,26] A diagnosis of maternal HCA (MHCA) was based on neutrophil infiltration at or below the chorionic plate; fetal HCA (FHCA) was identified by neutrophil invasion of veins or arteries in the chorionic plate and/or of the umbilical CB vessels.[3] Chronic inflammation was diagnosed in placentas with lymphocytic infiltration of the chorionic villi (*chronic villitis*), chorioamniotic membranes/plate (*chronic chorioamnionitis*), or basal plate (*chronic deciduitis*).[27] Gestations were defined as "controls" in placentas without evidence of HCA or chronic chorioamnionitis, or other significant pathology. Medical records were reviewed for maternal or fetal evidence of IAI or clinical chorioamnionitis.[28,29]

Blood Sample Collection

Anticoagulated (citrate phosphate) maternal blood samples were obtained from pregnant women by peripheral venipuncture within 24 hours prior to delivery. For cord blood (CB) samples, anticoagulated blood was aspirated from the placental umbilical vein (cleansed of maternal blood) immediately following delivery. Whole blood was processed for flow cytometric analysis and for the collection of plasma aliquots as described.[13] Plasma samples were stored at −80°C until batch analysis. For *in vitro* studies, anonymous CB samples (collected less than 12 hours postdelivery) were obtained from the SSM Health St. Louis cord Blood Bank.

Flow Cytometric Analyses of Patient Samples

Multiparameter flow cytometric analyses of antibody-stained whole blood samples were used to identify specific immune cell subsets. Briefly, samples stained with fluorochrome-labeled mAb or type-specific immunoglobulin G (IgG) controls were acquired within 24 hours of staining using a BD LSR-II Flow Cytometer, and were analyzed with the FlowJo 7.2.2 software (Tree Star, Ashland, Oregon, USA), as previously described.[13] Within the viable gated $CD3^+$ lymphocyte population, Th17 cells were identified in $CD4^+$ cells with surface expression of $CD161^+$ (progenitor Th17 cells [pTh17])[30] or both $CD161^+$ and $CCR6^+$ (mature Th17 cells [mTh17]) (7). The T regulatory (Treg) cells were identified in $CD4^+$ cells expressing the $CD25^{hi}CD127^{lo}$ phenotype.[31] The $TCR\gamma\delta^+$ T cells were identified within the gated $CD3^+$ cell population.[32] Furthermore, the Th17:Treg ratios were determined by calculating the ratios of pTh17 or mTh17 cell frequencies, respectively, to those of Treg cells.

Determination of Plasma Calgranulins

Calgranulin levels were determined in batched duplicate plasma samples using commercial ELISAs (CircuLex S100A8/MRP8, catalog No. CY-8061; CircuLex S100A12/EN-RAGE, catalog No. CY-8058; Circulex; MBL International Corporation, Woburn, Massachusetts, USA). Readings (405 nm) were compared against an internal standard curve, and the concentrations of S100A8 or S100A12 in each sample were calculated by plotting against a four-parameter logistic equation. Assay limits for detection were: S100A8,

43.4 pg/mL; S100A12, 8.2 pg/mL. Due to variability in plasma sample volumes, S100A8 and/or S100A12 levels were not determined in all subjects.

Th17 Cell Propagation in CB Cultures

For these studies, CB $CD4^+$ cells were purified from mononuclear cells, as we described.[33] Briefly, $CD4^+$ T cells were isolated by negative selection (EasySep™ Human Naïve $CD4^+$ T Cell Isolation Kit (Catalog 19555), STEMCELL Technologies, Vancouver, Canada) according to the manufacturer's instructions. Purified $CD4^+$ cells (2×10^6 cells) were suspended in CTCM (2 mM glutamine, 50-μM β-mercaptoethanol, 10% heat-inactivated human AB type serum, 100-U penicillin/100 μg streptomycin/mL) and cultured either in CTCM alone, or in CTCM containing a Th17-propagating cocktail (10 ng/mL: Interleukin-1β (IL-1β), IL-6, IL-23; 3 ng/mL: TGFβ) or varying concentrations of rh-S100A12. Cell suspensions were added to 24 well plates coated with anti-CD3 Ab (2 μg/mL) and in the presence of IL-2 (50 U/mL) at 37°C, 5% CO_2. Following a 72-hour culture, cells were harvested and stimulated for intracellular staining, including viability, as described.[33] Samples were acquired within 24 hours of staining using a 16-color BD LSRII flow cytometer. Acquired samples were analyzed using the FlowJo 7.2.2 software (Tree Star, Ashland, OR). Within the gated $CD4^+$ T cell population, Th1 cells were identified by the intracellular expression of the nuclear factor, Tbet, and Th17 cells were identified by expression of the nuclear factor, RORγt, or IL-17A. Tregs were identified in $CD4^+$ cells with intracellular expression of the nuclear factor, Foxp3.

Antibodies and Reagents

Fluorochrome-labeled mAb (all, Becton-Dickinson, Franklin Lakes, New Jersey, USA) were used for surface staining: CD3-FITC (clone SK7), CD4-Alexa Fluor 700 (RPA-T4), CD25-PE (2A3), CD45-V450 (HI30), CD127-BV650 (HIL-7R-M21), CD161-APC (DX12), CD196-PerCPCy5.5 (IIA9), and TCRγδ-BV605 (B1). The vital stain, Live/Dead Aqua, was purchased from Invitrogen/Thermo Fisher Scientific (Waltham, MA; catalog No. L34957). Recombinant human (rh) cytokines were purchased from BD Biosciences, San Jose, CA (rhIL-2), Peprotech, Inc., Rocky Hills, New Jersey, USA (rhIL-1β, rhIL-6), and R&D Systems, Minneapolis, Minnasota, USA (rhIL-23, rhTGFβ, rhS100A12).

Statistical Analyses

Experimental data were analyzed using the non-parametric Kruskal–Wallis test for intragroup comparisons across conditions; the non-parametric Mann–Whitney *U* test or independent Student's *t*-test for comparisons of unpaired data; and the non-parametric Wilcoxon rank test for paired data analyses (Prism v7; GraphPad Software, La Jolla, California, USA). Demographic data were compared using the Mann–Whitney *U* test or independent Student's *t*-test for continuous data, or Fisher's exact test for categorical data using Statistical Package for the Social Sciences (SPSS) (v23; IBM, Armonk, New York, USA) or Prism. Correlations between variables were calculated using the Pearson correlation coefficient; $p < 0.05$ was considered significant.

Results

Subject Characteristics and Demographics

We studied 47 women in preterm labor who were enrolled at the time of their admission to labor and delivery, and their delivered neonates. Placental analyses identified 37 gestations with HCA (12 MCHA; 25 FHCA). Seven placentas were unaffected by HCA or other identified placental pathology, and served as controls. Placentas of three gestations were diagnosed with chronic inflammation only (villitis or deciduitis); these were analyzed separately from the HCA or control groups. Key baseline maternal and perinatal characteristics, including clinically suspected IAI, were not different between groups (Table 1). Neonates with HCA were delivered at earlier gestational ages relative to controls (Table 2), especially in the presence of FHCA (29.4 ± 3.1 weeks, $p = 0.02$); however, no age differences were observed between MHCA and FHCA gestations. Birth weights were lower in HCA relative to controls, particularly in the presence of FHCA (1471 ± 542 gm, $p = 0.01$). In contrast, the three neonates with chronic inflammation had higher birth weights (2166 ± 465 gm) relative to CB with any HCA ($p = 0.02$) and FHCA ($p = 0.01$). No differences were observed between groups for the remainder of neonatal outcomes, including early- or late-onset sepsis.

Table 1: Maternal characteristics

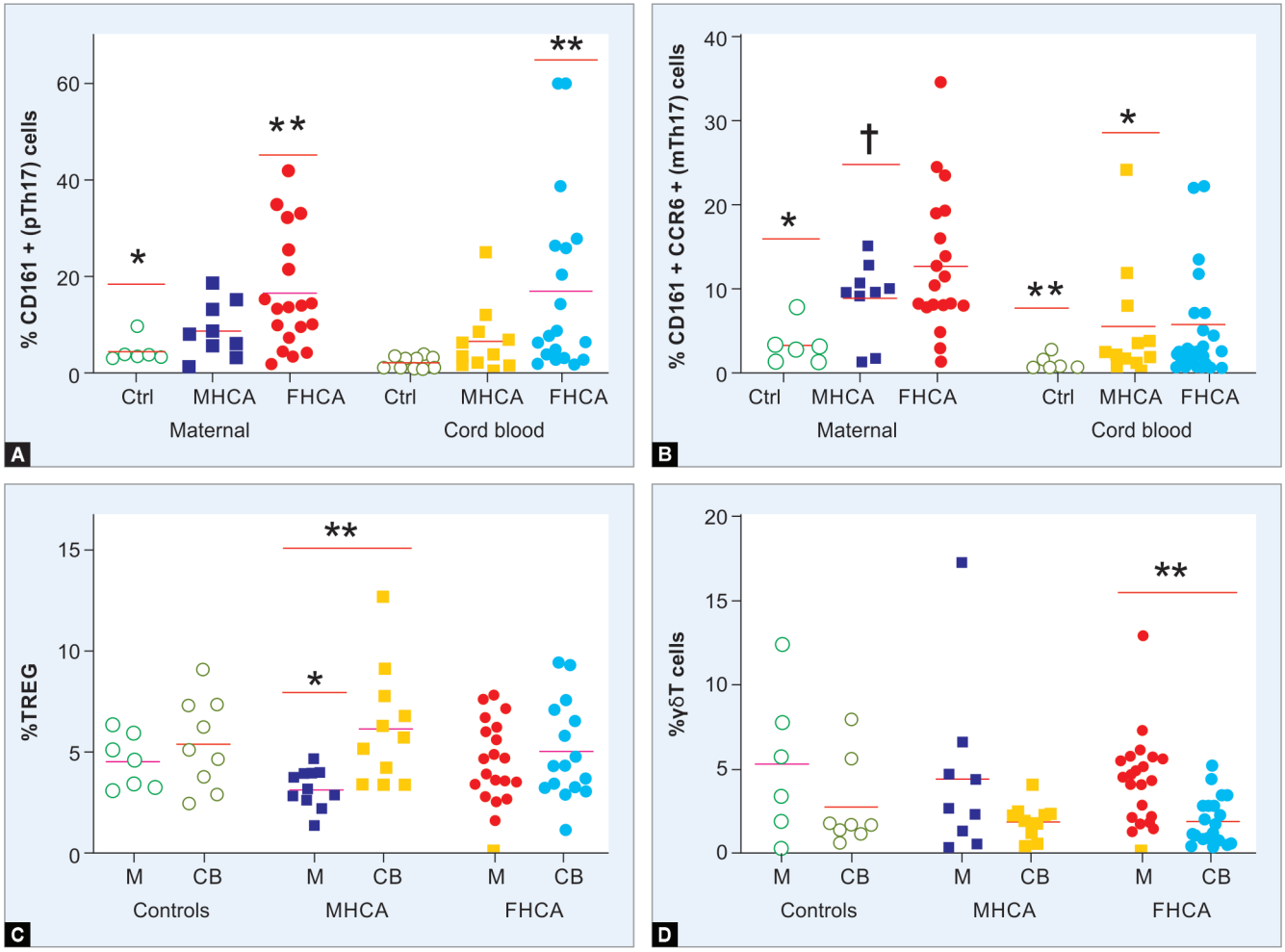
Parameter	*HCA (n = 37)*	*Controls (n = 7)*	*p-value*
Age (year)	26.8 ± 6.5	26.1 ± 7.0	0.80
BMI (kg/m^2)	30.2 ± 6.8	30.6 ± 5.9	0.86
African–American	19 (51%)	3 (43%)	>0.99
Caucasian	18 (49%)	4 (57%)	>0.99
Multiparous	22 (59%)	6 (86%)	0.39
Prior preterm delivery	9 (24%)	4 (57%)	0.17
History of smoking	8 (22%)	3 (43%)	0.34
PPROM	31 (84%)	6 (86%)	>0.99
Suspected IAI	6 (16%)	1 (14%)	>0.99
Antenatal antibiotics	37 (100%)	7 (100%)	>0.99
Antenatal steroids	37 (100%)	7 (100%)	>0.99

Data are shown either as mean ± standard deviation (SD) or as *n* (%). BMI, body mass index; IAI, intraamniotic infection; PPROM, prolonged preterm rupture of membranes

Table 2: Neonatal outcomes

Parameter	*HCA (n = 37)*	*Controls (n = 9)*	*p-value*
Gestational age at delivery (weeks)	29.9 ± 3.0	31.8 ± 2.6	0.04
Birth weight (gm)	1546 ± 612	1898 ± 533	0.03
SGA	2 (5%)	0 (0%)	>0.99
EOS	8 (22%)	1 (11%)	0.66
LOS	3 (8%)	0 (0%)	>0.99
IVH	3 (8%)	0 (0%)	>0.99
NEC	2 (4%)	0 (0%)	>0.99
BPD	5 (14%)	1 (11%)	>0.99
Death	4 (11%)	0 (0%)	0.57

Data are shown as either mean ± SD or as *n* (%). Death at delivery or at any time prior to hospital discharge. BPD, bronchopulmonary dysplasia; EOS, early-onset sepsis; IVH, intraventricular hemorrhage; LOS, late-onset sepsis; NEC, necrotizing enterocolitis; SGA, small for gestational age

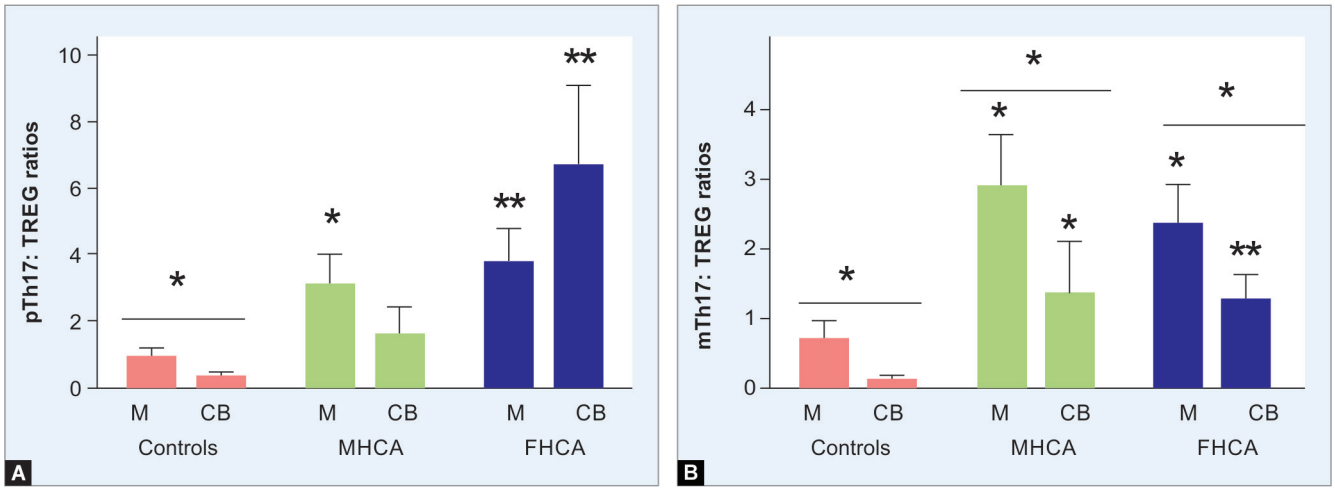


Figs 1A to D: Immune cell frequencies. Gated $CD4^+$ cells in maternal peripheral blood and CB (cord blood) samples from preterm gestations with HCA or from unaffected preterm controls were analyzed for circulating frequencies of Th17 and Treg cell populations by multi-parameter flow cytometric analysis. The analysis of γδ T cell populations was performed within gated $CD3^+$ cells. Each symbol represents a single subject. Horizontal bars represent mean ± SEM. (A) Progenitor (p)Th17 cells. Mean frequencies of $CD4^+CD161^+$ populations. Maternal (M)-CB pairs: Controls (Ctrl), $n = 6$; MHCA, $n = 11$; FHCA, $n = 19$. *$p < 0.05$ vs Ctrl; **$p < 0.01$ vs Ctrl; (B) Mature (m) Th17 cells. Mean frequencies of $CD4^+CD161^+CCR6^+$ populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 19$. *$p < 0.05$ vs Ctrl; **$p < 0.01$ vs Ctrl; $^{\dagger}p < 0.001$ vs Ctrl; (C) Treg cells. Frequencies of $CD4^+CD25^{hi}CD127^{lo}$ Treg populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 16$. *$p < 0.05$ vs Controls; **$p < 0.01$, M vs CB; (D) γδ T cells. Mean frequencies of $CD3^+TCR^+\gamma\delta^+$ cell populations. M-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 18$. **$p < 0.01$, M vs CB

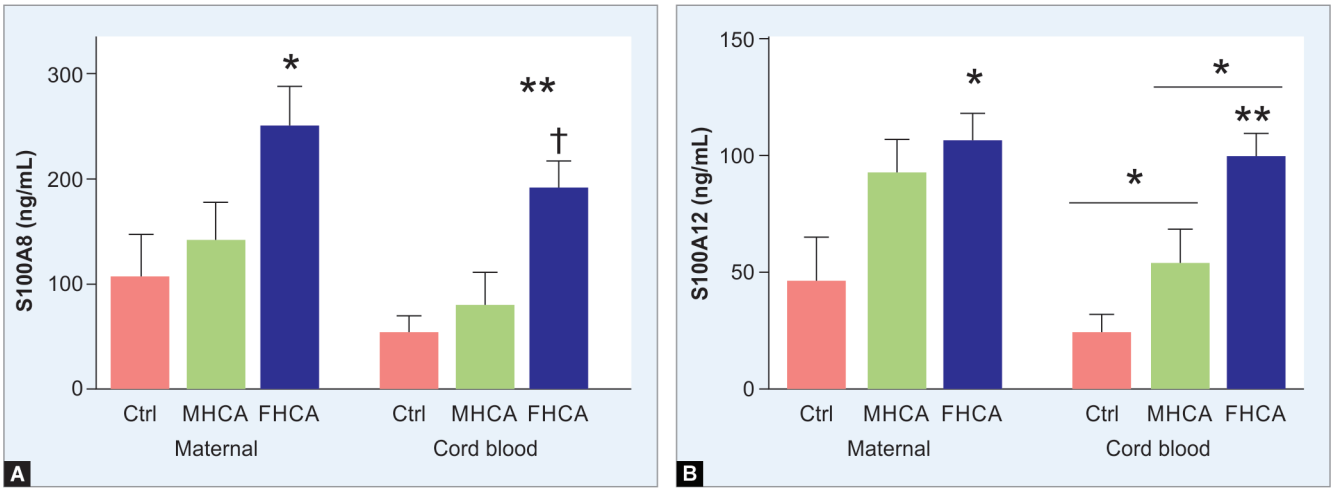
Immune Cell Responses

In flow cytometric studies of maternal peripheral blood and preterm CB, we examined the proportions of Th17 cells subsets with progenitor or mature phenotypes, as these have been variably linked to the pathogenicity of chronic inflammatory disorders.[7,34–36] We determined the highest frequencies of progenitor (p)Th17 cells in FHCA gestations for both mothers and in CB relative to controls, while these were significantly elevated only for mothers in MHCA (Fig. 1A). In paired comparisons, pTh17 cell frequencies were higher in mothers in control gestations ($p = 0.02$ vs CB); frequencies were similar between mothers and in CB with MHCA ($p = 0.42$) or FHCA ($p = 0.61$). In studies of mature (m)Th17 cells, we also observed the highest maternal and CB frequencies in FHCA gestations, similar to our observations for pTh17 cell subsets (Fig. 1B). Maternal and CB mTh17 cell frequencies were also both elevated in MHCA relative to control gestations. In paired studies, maternal mTh17 cell frequencies were higher than in the CB of their neonates in FHCA gestations; however, frequencies were not significantly different between pairs in MHCA ($p = 0.28$) or control ($p = 0.06$) gestations.

Quantitative alterations in Treg cells, which have the capacity to suppress Th17 responses,[37] have been reported in pregnancy-related inflammatory disorders, including in women with preeclampsia.[15] We examined frequencies of Treg cells in paired maternal-CB blood samples in gestations with or without HCA (Fig. 1C). We observed lower circulating Treg cell frequencies in mothers with MHCA ($p < 0.05$ vs controls), while no differences in CB Treg frequencies were determined between HCA and control gestations. In paired comparison studies, maternal Treg cell frequencies were also lower compared with CB in MHCA gestations but were similar to CB Treg cell frequencies in gestations with FHCA ($p = 0.89$) or in controls ($p = 0.05$).



Figs 2A and B: The Th17:Treg ratios. The Th17:Treg ratio was calculated by dividing individual frequencies of pTh17 or mTh17 cells by Treg cell frequencies. Maternal (M)-CB pairs: Ctrl, $n = 7$; MHCA, $n = 11$; FHCA, $n = 13$. (A) pTh17:Treg ratio. *$p < 0.05$, M vs CB; $p < 0.05$ vs Controls; **$p < 0.01$ vs Ctrl; $p < 0.01$, M vs CB; (B) mTh17:Treg ratio. *$p < 0.05$, M vs CB; $p < 0.05$ vs Ctrl; **$p < 0.001$ vs Ctrl



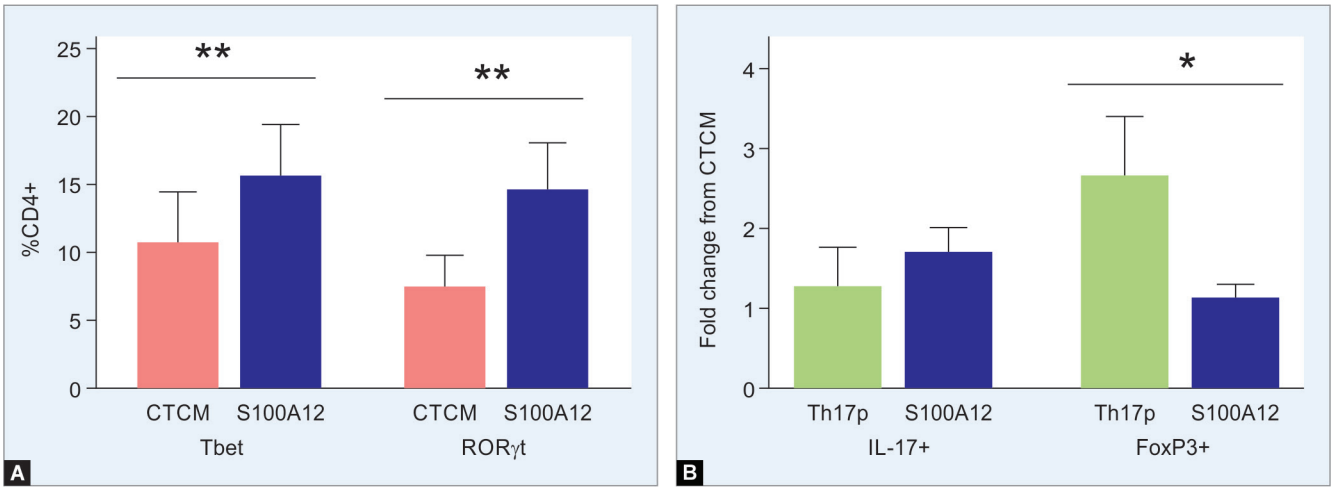
Figs 3A and B: Maternal and CB S100A8 and S100 A12 plasma levels. Plasma levels of S100A8 or S100A12 were determined in maternal (M)-CB pairs from HCA or control gestations. (A) S100A8 levels. Ctrl, $n = 9$; MHCA, $n = 10$; FHCA, $n = 18$. *$p < 0.05$ vs Ctrl; **$p < 0.01$, MHCA vs FHCA; †$p < 0.001$ vs Ctrl; (B) S100A12 levels. Ctrl, $n = 9$; MHCA, $n = 10$; FHCA, $n = 20$. *$p < 0.05$ vs Ctrl; MHCA vs FHCA; **$p < 0.001$ vs Ctrl

Gamma-delta T cells represent a lymphocyte subset that may contribute to inflammatory pathology in part through the release of IL-17.[38] To more fully define Th17-type responses in HCA, we compared maternal and CB γδ T cell frequencies in affected and control gestations (Fig. 1D). No differences in γδ T cell frequencies were determined for either mothers or in CB for any HCA condition relative to controls. However, in pairwise assessments, higher γδ T cell frequencies were determined in mothers vs CB in FHCA, while frequencies were similar between pairs in both MHCA ($p = 0.17$) and control gestations ($p = 0.19$).

The Th17:Treg ratio has been correlated with disease severity in a variety of inflammatory disorders.[39] We observed elevated pTh17:Treg ratios for both mothers and in CB in FHCA vs control gestations, while only maternal pTh17:Treg ratios were elevated in MHCA gestations (Fig. 2A). We also determined elevation of mTh17:Treg ratios for mothers and in CB in gestations with either HCA condition vs relative controls (Fig. 2B). In paired analyses, pTh17:Treg ratios were not different between mothers and CB for a specific HCA condition (MHCA, $p = 0.07$; FHCA, $p = 0.55$), while maternal ratios were higher than CB ratios in controls (Fig. 2A). In contrast, mTh17:Treg ratios in mothers were higher than in the CB of their neonates for any gestational condition (Fig. 2B). In analyses that compared controls with combined MHCA and FHCA ratios (any HCA), maternal and CB ratios were higher in gestations with any HCA (MHCA + FHCA) for both Th17 subsets: pTh17 (maternal, $p = 0.01$, CB, $p = 0.02$); mTh17 (maternal, $p = 0.04$, CB, $p = 0.03$).

S100A8 and S100A12 Levels

As calgranulins and other S100 proteins have been linked to Th17-mediated inflammatory disorders (16, 17), we performed comparison studies of maternal and CB calgranulin levels in HCA or control gestations (Fig. 3). In gestations with FHCA, S100A8 levels in CB were increased three-fold relative to controls while maternal levels were doubled (Fig. 3A). In contrast, in MHCA gestations, S100A8 levels were not elevated for either mothers or CB. In studies of S100A12 expression, in FHCA gestations, CB levels were quadrupled relative



Figs 4A and B: The rh-S100A12 and Th17 propagation in term CB cultures. $CD4^+$ T cells isolated from banked CB were incubated for 72 hours in the presence of complete medium (CTCM) containing either S100A12 (1000 ng/mL) or Th17-propagating cocktail (Th17p), then analyzed by flow cytometry. Data represent the results of about four to six individual studies. *$p < 0.05$; **$p < 0.01$. (A) rh-S100A12 vs CTCM. rh-S100A12 induced greater propagation of $Tbet^+$ and $ROR\gamma t^+$ CD4 cells relative to culture with CTCM alone; (B) S100A12 vs Th17 propagating cocktail (Th17p). The induction of $CD4^+FoxP3^+$ cells by rh-S100A12 alone was only half of those determined in the presence of Th17p

to controls, while maternal S100A12 levels were nearly twice those of controls (Fig. 3B). In MHCA gestations, elevation of S100A12 levels were significantly elevated only for CB. Paired analyses showed similar expression levels of S100A12 between mothers and preterm CB for all gestational conditions (controls, $p = 0.21$; MHCA, $p = 0.16$; FHCA, $p = 0.81$). Paired CB comparisons of S100A12 levels and Th17 cell frequencies showed a correlation for pTh17 frequencies ($r = 0.62$, $p < 0.02$), with a relationship also observed for mTh17 cell frequencies ($r = 0.44$ $p < 0.02$).

rhS100A12 Promotes Th17 Cell Propagation in CB Cultures

The prominent CB S100A12 levels observed in association with fetal inflammation in this study led us to hypothesize that S100A12 might serve as a "transducer" between fetal neutrophil and Th17 responses. In preliminary *in vitro* studies, coculture of term CB $CD4^+$ cells with rhS100A12 resulted in a higher proportion of cell populations expressing the nuclear factor, $ROR\gamma t$ (a Th17 cell marker), as well as the Th1 nuclear factor, Tbet (Fig. 4A), compared to $CD4^+$ cells cultured in media alone. Cultures containing either rh-S100A12 alone or only a potent Th17-propagating cytokine cocktail induced $IL\text{-}17^+$ $CD4^+$ cells to a similar degree ($p = 0.31$) (Fig. 4B). However, the S100A12 effect appeared to be specific for Th17 cell induction, as increased Treg ($CD4^+$ $FoxP3^+$) cell frequencies were observed in cultures containing a Th17-propagating cocktail but not in the presence of S100A12 alone (Fig. 4B).

Discussion

The primary goal of the present study was to determine maternal Th17-type responses relative to those in the CB of their neonates in preterm gestations with HCA. We determined concurrently elevated circulating expression levels of Th17 cells in whole blood and the plasma Th17-associated calgranulins, S100A8 and S100A12, in both mothers and in preterm CB, particularly in the presence of fetal inflammation. To our knowledge, this is the first report describing these combined Th17-related responses in both mothers and in preterm CB in HCA gestations.

Our findings provide added evidence supporting the role of maternal–fetal crosstalk mechanisms and extend existing information regarding Th17 responses in pregnant women and in neonates with HCA gestations.[13,14,40] We observed elevations in circulating Th17 cell subset frequencies in both pregnant women in preterm labor and in the CB of their preterm neonates especially in gestations with fetal inflammation (FHCA). Notably, while elevated in both groups, mTh17 cell frequencies were higher in mothers relative to those in the CB of their neonates in gestations with fetal inflammation. This latter observation may reflect a specific maternal inflammatory response to fetal inflammation, as a strong association between elevated mTh17 cell responses and inflammatory status has been reported in other disorders.[41] Our results differ from a recent report showing higher maternal Th17 frequencies in term vs preterm gestations.[40] However, our results may reflect the focus of our study on the comparison of preterm gestations with a diagnosis of HCA vs those with absent placental or infectious pathology. That report also described elevated circulating maternal IL-6 levels in preterm gestations as a group, although whether maternal IL-6 levels correlated with placental inflammation was not clear (and fetal inflammation was not specifically identified). However, this finding is supportive of increased Th17-linked responses, as IL-6 is critical to the preferential propagation of Th17 cells over anti-inflammatory Treg cells.[42] In our studies of γδ T cells, an immune cell that is an important source of IL-17,[34] we found higher frequencies in mothers than in the CB of their neonates specifically in gestations with fetal inflammation. In light of an established association between Th17-type responses and inflammatory disorders,[25] these maternal Th17-type responses are consistent with the possibility that maternal exposure to fetal inflammation could set the stage for later maternal metabolic or cardiovascular disease.[43]

Enhanced Th17 responses have been ascribed to neuroinflammation in neonates,[9] and have been associated with the severity of chronic inflammation in adults.[39] Prominent circulating Th17 cell populations have also been observed in women with recurrent pregnancy loss and pre-eclampsia, gestational disorders also associated with inflammation.[44,45] Our present findings extend

previous observations of elevated Th17 responses in preterm neonates with HCA[13,14] and provide new evidence of a potential involvement of maternal Th17 cells in its pathogenesis.[46,47] In addition, the observed imbalances between Th17 cells and Tregs could enhance tissue Th17 responses that amplify the inflammatory cascade[8,48] in mothers, in neonates, or in both. Some data suggest that pathologic Th17 cells contribute to preterm labor through processes involving fetal immune activation against maternal antigens.[12,49] In addition, imbalances in Th17 and Treg cell expression levels (such as in this study and in other[13,14] studies) as well as Th17-calgranulin interactions have been observed in conjunction with immune rejection processes.[15,17] Notably, rejection has been identified as a potential mechanism associated with preterm birth[22,49] In addition to its possible role in preterm delivery, intrauterine Th17-mediated inflammation could "imprint" the immune system of the developing fetus, leading to altered postnatal responses.[50,51] Taken together, our present observations highlight a need for improved understanding of the roles of pathogenic Th17 processes and Th17 cell heterogeneity to the development of placental inflammation.[8] Such information could guide the development of novel prenatal therapeutic approaches,[52] for example, by targeting elements of the Th17 pathway.[53]

Our studies included analyses of circulating maternal and CB levels of the calgranulin proteins, S100A8 and S100A12. We found marked elevations of plasma calgranulin levels in mothers and especially in the CB of their preterm neonates in the context of fetal inflammation. Our findings are consistent with the increased S100A12 blood levels previously reported in a small subset of neonates born after HCA,[21] as well as recently described neonatal monocyte and blood expression levels of S100 proteins in chorioamnionitis.[20] While the role of calgranulins in the pathogenesis of fetal inflammation has not been discerned, evidence suggests a link between inflammatory neutrophils, a driving force in this disease process[54,55] and their contribution to circulating S100 proteins.[56] Pertinently, neutrophils can promote Th17 cell propagation and function,[33,57] a process that may involve neutrophil-derived calgranulin mediators (in this study and in other[58,59] studies). Conversely, calgranulins modulate inflammatory responses by promoting neutrophil production and activation.[60,61] Additionally, crosstalk between neutrophils and Th17 cells can amplify the inflammatory cascade.[62] Our finding that rhS100A12 also promoted the expression of Tbet, a canonical nuclear transcription factor for Th1 cells,[63] is consistent with the Th1 polarization bias observed in exometabolomic studies of HCA-exposed preterm neonates.[64] Our preliminary observations in the context of existing data hint at feed-forward mechanisms involving interactions between neutrophils, calgranulins, and Th17 cells in the pathophysiology of fetal inflammation. However, our findings are based on *in vitro* studies that targeted healthy CB $CD4^+$ cells of term gestations. Future studies involving preterm CB cells and *in vivo* models may provide important clues to understanding whether these "pieces fit the puzzle" of mechanisms that drive fetal inflammation and preterm birth and as well as postpartum maternal inflammatory disease.[65]

Our observations suggest an attributable risk of HCA in association with elevated Th17 cell frequencies and calgranulin levels, despite the limited size of our study sample. Our findings also support the potential utility of calgranulin levels in identifying clinically "silent" fetal inflammation, an important task given its association with preterm delivery and adverse neonatal outcomes.[20,66] Such information could also facilitate anticipatory clinical management of HCA and guide effective postpartum and postnatal interventions.[52,67,68] However, studies in much larger, diverse populations are clearly warranted to define the use of calgranulins in this context.

A major strength of this study lies in its prospective design with stringent inclusion and exclusion criteria that minimize the presence of confounding factors associated with other perinatal inflammatory disorders.[69] Thus, despite our limited sample size, the significant intergroup differences in Th17 responses that we now report in maternal-CB dyads with fetal inflammation support the biological relevance of our findings. However, our study was neither powered to identify infectious etiologies nor to specifically correlate our findings with adverse maternal or neonatal outcomes.

In summary, the enhanced Th17-linked response patterns observed in pregnant women parallel those in their preterm neonates especially in the context of fetal inflammation. These findings provide compelling supportive evidence of maternal–fetal crosstalk mechanisms that may influence gestational inflammatory or immune processes.[27] While our understanding of perinatal Th17 responses in HCA continues to evolve, their contributions to maternal and neonatal health and disease remain critical knowledge gaps that will benefit from continued investigations in this area.

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Author Contributions

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REVIEW ARTICLE

Sodium and Growth in Preterm Infants: A Review

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Abstract

Aim: This article is intended to review the relationship between sodium homeostasis and growth, outline reasons why preterm infants may become sodium deficient, and share data from our group and others regarding the potential benefits of dietary sodium supplementation.

Background: Despite tremendous efforts over the past 20 years to optimize neonatal nutrition, postnatal growth failure in preterm infants remains a significant problem. Compelling associations have been identified between in-hospital growth failure and cardiometabolic and neurodevelopmental disorders, heightening the need to further identify the optimal nutritional needs of preterm infants.

Results: The impact of sodium deficiency may have on somatic growth is poorly studied and reported upon within the human literature. In contrast, animal studies dating back almost 100 years highlight the nutritional importance of dietary sodium. Sodium homeostasis during early postnatal life is understudied and underappreciated by neonatologists.

Conclusion: Insufficient sodium intake during early life is likely a critical yet underappreciated contributor to growth failure. Total body sodium depletion may be an important risk factor driving complications of premature birth.

Clinical significance: Increased awareness of sodium homeostasis in preterm infants may improve outcomes in this population. Sodium intake recommendations are provided based on the interpretation of currently available literature.

Keywords: Growth, Human, Postnatal, Premature, Preterm, Review, Sodium.

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Aim

From soon after admission to a neonatal intensive care unit until often near the time of hospital discharge, clinicians prescribe the amount of protein, lipid, carbohydrate, and fluid an infant receives. A primary focus of care is achieving optimal growth of patients, with the knowledge that postnatal growth failure is linked with increased risk of morbidity, including neurodevelopmental impairment.[1,2] Thus, extensive study has occurred regarding the optimal intake of various components of nutrition. One area of parenteral and enteral nutrition that has been overlooked is a mineral intake and in particular sodium intake. For almost 100 years, the importance of adequate dietary sodium intake to achieve maximal growth has been recognized.[3] Subsequent studies in animals and human infants have repeatedly demonstrated that inadequate sodium intake early in life impairs growth and may impact other physiological functions. The purpose of this review is to highlight the importance of sodium intake early in life, the gaps in our understanding of how to identify sodium deficiency, and our lack of recognition of what sodium requirements for preterm infants over the first few months of life.

Background

Why would a Preterm Infant become Sodium Depleted?

Newborn infants are in a precarious state of sodium balance. For term infants who receive strictly breastmilk for the first months of life, sodium intake is obviously limited to that within breastmilk. Beyond the first postpartum week following term delivery, the sodium content in breastmilk is typically no more than 10 mEq/L.[4] Assuming a daily intake of 150–175 mL/kg of milk, an infant would receive approximately 1.5–1.75 mEq/kg/per day of sodium. Since >98% of ingested sodium is likely absorbed and sweat production is minimal, sodium losses during infancy are primarily urinary. The mature kidney displays redundant sodium transport systems that allow for a high degree of renal tubular sodium reabsorption (thus a low fractional excretion of sodium and urinary sodium concentration). Assuming a urine production of 50 mL/kg/d (approximately 2 mL/kg/hour) and a urine sodium concentration of 10 mEq/L (a conservative value assuming a well-functioning kidney), urine sodium losses average 0.5 mEq/kg/d. Thus, the net sodium balance, assuming no stool or skin losses, would be 1–1.25 mEq/kg/d. If one further assumes that infant growth averages 25 g/d, with total body water at this stage of development is being approximately 70% of body weight, and with equal distribution of intracellular and extracellular compartments, an infant requires 1.225 mEq/d of sodium for growth. This calculation is as follows: (A) 25 g/d growth, of which 70% is water = 17.5 g (mL); (B) 50% of this water is (8.75 mL) extracellular; (C) Sodium concentration

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of extracellular water is 140 mEq/L; (D) 8.75 mL × 140 mEq/L = 1.225 mEq. Thus, any decrease in sodium intake or increase in sodium losses may put the infant at risk for sodium deficiency and suboptimal growth.

For the preterm infant, the mother's milk is insufficient to meet the sodium needs for growth. Milk samples obtained over the first month after birth from mothers delivering at approximately 28 weeks gestation contained approximately 30–35 mg/dL of sodium (1.3–1.52 mEq/dL).[5] Assuming an intake of 160 mL/kg/d, this would result in a sodium intake of only 2.08–2.42 mEq/kg/d. As will be discussed below, this sodium intake is insufficient due to the higher obligate urine sodium losses in preterm compared to term infants. Donor breastmilk, even with added commercial fortifiers is also insufficient to meet the sodium needs of the preterm infant. Perrin et al. measured the sodium content of 300 samples of donor human milk and estimated the sodium content resulting from fortification with 3 different human milk fortifiers and feeding volumes of 160 mL/kg/d.[6] They reported a sodium content of approximately 100 mg/L (range 40–570) (4.35 mEq/L, range 1.74–24.78 mEq/L in donor human milk samples. Depending on the type of fortifier used (Similac Human Milk Fortifier Hydrolyzed Protein Concentrated Liquid, Abbott Laboratories), bovine-based fortifier to 24 kcal/ounce (Enfamil Liquid Human Milk Fortifier High Protein; Mead Johnson), and human-milk-based fortifier (Prolacta + 6; Prolacta Bioscience), the expected mean sodium intake from donor human milk was 1.6–3.4 mEq/kg/d.

We have recently argued that term babies are capable of protecting themselves from a sodium deficit in the first few months of life (in the absence of significant pathology) by utilizing stores of osmotically inactive sodium which develop in utero.[7] These stores result from sodium binding negatively charged glycosaminoglycans with skin and other tissues. Studies from rats demonstrate that such stores can be mobilized for growth early in life and during conditions of sodium depletion.[8] Using data from previously published literature, we estimated the term fetus may 'store" up to 80 mEq Na, the vast majority of these stores being accumulated during the last 10 weeks of gestation. The term newborn may then these stores as necessary to achieve optimal growth in the first few months after birth. The absence of these osmotically inactive sodium stores in the preterm infant may be one of the risk factors for the development of sodium deficiency, and thus postnatal growth failure in this vulnerable population.

In contrast to the mature kidney, kidneys from preterm infants have a limited capacity for sodium reabsorption due to the immaturity of renal tubular sodium transporters (recently reviewed by Gattineni and Baum).[9] In preterm infants, fractional excretion of sodium (FENa) and urine sodium excretion (UNaV) are inversely associated with gestational age at birth and postnatal age.[10,11] Longitudinal study of preterm infants reveals FENa exceeded 6% in infants <28 weeks of gestation on the day of life 3, decreasing to about 4% by the end of the first week of life and to 2% at a month of age.[12] We previously calculated expected urinary sodium losses in preterm infants across a range of gestational and postnatal ages.[13] Even at 6 weeks of postnatal age, infants 23–27 weeks gestational age are estimated to lose 5–5.5 mEq/kg of sodium per day, likely exceeding the sodium intake of many preterm infants at this postnatal age. Losses at earlier postnatal ages were greater. More recently, we confirmed these findings by longitudinal examination of sodium balance in infants 22–23 weeks of gestation from 2 to 10 weeks of postnatal age.[14] Urine sodium losses exceeded 6 mEq/kg/d until 31 weeks postconceptional age. These losses were not driven by high sodium intakes as sodium balance was not significantly positive until 33 weeks of post-conceptional age. Serum sodium values for the cohort remained in the normal range despite a negative sodium balance, while no significant relationship was identified between sodium intake and serum sodium values. This finding emphasizes that serum sodium values are more reflective of body water homeostasis rather than sodium balance and that serum sodium values cannot be the sole factor in determining the prescription of sodium to preterm infants.

Though not extensively studied, dysregulated hormonal responses appear to contribute to high renal sodium losses early in life. In term infants, cord blood aldosterone and renin levels are significantly greater than paired maternal levels, though urine sodium losses are high and there is an absence of correlation between urine aldosterone and urine potassium concentrations and urine Na^+/K^+ ratio.[15] Thus, despite strong activation of the renin-aldosterone system, partial aldosterone resistance appears to be present. In a separate study involving preterm infants and using urine aldosterone concentration and urinary Na^+/K^+ ratio as an index of renal aldosterone sensitivity, Martinerie et al. concluded preterm but not term infants display aldosterone sensitivity.[16]

However, because the activity of the renin-angiotensin-system may be impacted by numerous factors early in life, and urine aldosterone excretion may not truly reflect aldosterone secretion, the conclusions for this study have been refuted.[17] Atrial natriuretic peptide (ANP) may also play a role in sodium homeostasis early in life. In preterm infants with mean gestational ages of approximately 31 weeks, mean daily Na intakes of 1.4–1.8 mEq/kg/d from weeks 1–5 after birth resulted in sustained levels of plasma ANP whereas infants receiving sodium intakes of 4.6 +/-1.0 mEq/kg/d demonstrated a steady decrease in ANP levels.[18] However, Shaffer et al. found no correlation between plasma ANP concentrations and sodium excretion or fractional sodium excretion.[19] Other investigators have demonstrated that ANP levels are elevated during postnatal adaptation, are greater in preterm than term infants, and may be impacted by respiratory status.[19–21] Thus, whether ANP significantly affects renal function during the postnatal period remains unclear.

What Are the Potential Mechanisms by Which Sodium Depletion Results in Growth Failure?

Studies in young animals have been revealed an understanding the need for sufficient sodium intakes early in life. In young growing rats, sodium-deficient diets impair weight and length gain, impair bone growth, diminish nitrogen retention, and decrease muscle protein synthesis.[22,23] Sodium supplementation to sodium-depleted animals restore normal rates of weight and length gain and protein synthesis. Fine et al. placed weanling rats on diets with sodium intakes ranging from 30 to 900 μeq/day for 5 weeks.[24] Doses less than 300 μeq/day were associated with decreased weight gain, nitrogen accretion, and fat-free dry weight. Doses greater than 300 μeq/day were not associated with further weight gain. Importantly, total body water, as a percent of body weight, and serum sodium values were similar across groups at the end of the 5-week study, despite 30-fold differences in sodium intake. These findings suggest chronic differences in sodium intake do not impact total body water (i.e., water retention) and that serum sodium values may be normal even in the face of significant total body sodium depletion. Sodium-deficient animals ingested a greater amount of food per gram weight gain than animals on higher sodium diets, consistent

with alterations in energy efficiency (weight gain (grams)/energy absorbed.

The mechanisms by which sodium depletion may impair somatic growth remain to be fully elucidated. Haycock suggested that depletion of extracellular sodium decreases Na^+/H^+ antiporter activity, thus altering intracellular pH and the cell's ability to respond bind and respond to various growth factors.[25] Indeed, alkalinization of the cytoplasm by stimulation of the antiporter by mitogens appears necessary for cell proliferation.[26] Work from our own laboratory using a mouse model of early life sodium depletion similarly found impaired somatic growth when sodium intake was less than a critical value but not enhanced by excess dietary sodium.[27] Additionally, sodium depletion impaired energy efficiency (efficiency in which an animal uses absorbed energy for growth) but not digestive efficiency (efficiency of absorbing ingested energy) or caloric intake, suggesting energy expenditure is increased in association with sodium depletion. Preliminary findings from our laboratory using indirect calorimetry support the idea that total aerobic energy expenditure is increased in mice fed a low-sodium diet even after correction for body composition.[28] These findings provide strong evidence that early-life sodium supply impacts energy homeostasis and growth kinetics and prompts an increased focus on identifying optimal sodium supplementation for prematurely born and low-birthweight infants.

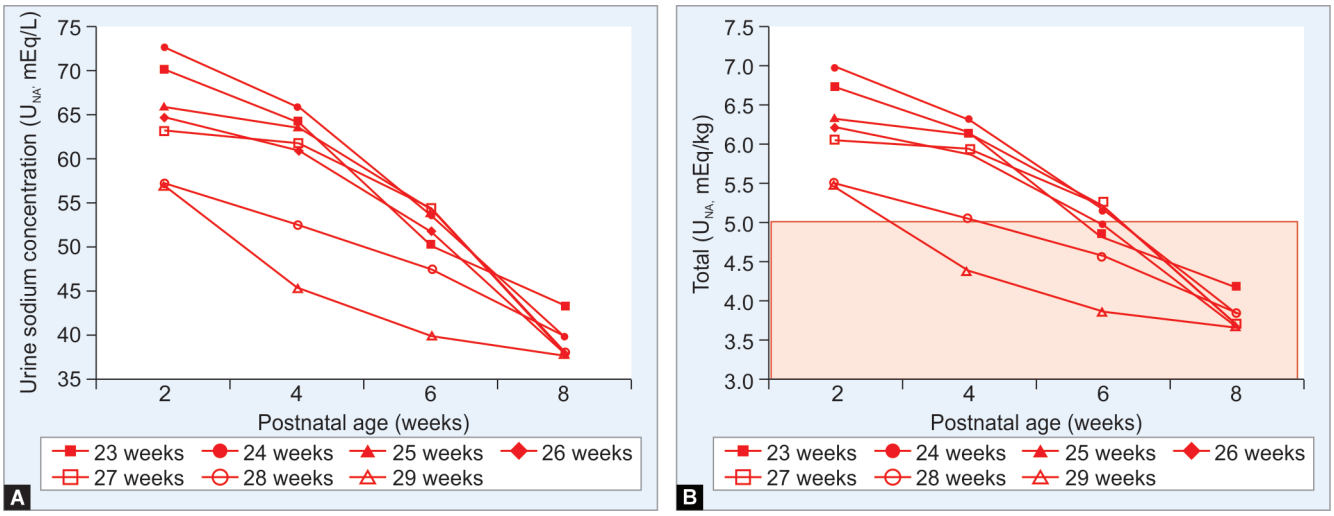
Does Sodium Supplementation Result in Improved Postnatal Growth in Preterm Infants?

Studies in preterm infants, though limited, support a beneficial effect of sodium supplementation above that is typically provided in the diet to optimize postnatal growth. Vanpée et al. randomized infants 29–34 weeks gestation to oral NaCl supplementation of 4 mEq/kg/d from 4 to 14 days of age or routine nutritional care, including enteral feedings and parenteral nutrition (n = 10 per group).[29] Average daily sodium intake over this period was 5.0 =/–0.9 mEq/kg/d in the supplemented group and 1.7 +/– 0.6 mEq/kg/d in control group. At two weeks of age, supplemented infants weighed more than birthweight (+5.8 +/–7.2%), whereas control infants had not regained birthweight (–1.6 +/– 4.6%). Fluid intake and urine output were similar between the control and supplemented infants, suggesting that weight gain was not a result of water retention. Al-Dahhan et al. similarly supplemented 22 infants born at 27–34 weeks gestation to a total daily sodium intake of 4–5 mEq/kg/d on postnatal days 5–11.[30] Compared to the control group (n = 24) with sodium intakes of slightly less than 2 mEq/kg/d during this period, the supplemented group showed increased weight gain. After 2 weeks of age, the supplemented group had a slight but significantly increased average daily sodium intake of approximately 0.8 mEq/kg /d compared to the control group, while continuing to display increased rates of weight gain. In a more recent study, Isemann et al. randomized infants <32 weeks gestation to receive 4 mEq/kg/d of Na, or placebo, from days of life 7–35, resulting in an average daily Na intake of 6.3 mEq/kg in the supplemented group and 2.9 mEq/kg in the placebo group.[31] Fifty-three infants were enrolled at an average gestational age of 28.5 weeks. NaCl was administered enterally (four times daily) if feedings equaled or exceeded 100 mL/kg/d. Unfortunately, only 29 infants completed the study related to death, transfer, and hospital discharge, approximately half <28 weeks of gestation. At 6 weeks of postnatal age, 79% of supplemented infants maintained their birthweight percentile (i.e., did not demonstrate postnatal growth failure) compared with only 13% in the placebo group. The difference in weight gain was particularly accentuated in the subgroup of infants born at <28 week's gestational age. Caloric intake was similar between groups, while sodium daily sodium intake for the 4-week intervention period averaged 6.3 ± 0.4 vs 2.9 ± 1.0 mEq/kg/d in the supplemented vs control infants, respectively. Collectively, these studies suggest that in the absence of sodium supplementation, human milk and currently available formulas fail to provide the nutritional sodium requirements to achieve optimal growth in preterm infants.

Table 1: Recommended sodium intake (mEq/kg/day) according to gestational and postnatal ages

	Postnatal age, days				
Gestational age, weeks	*1–2*	*7*	*14*	*28*	*56*
22–25	0–2	6–9	6–9	5–8	4–6
26–28	0–2	5–8	5–8	4–7	3–5
29–31	0–2	4–7	4–7	3–6	3–5
32–36	0–2	3–5	3–5	3–4	2–4

We previously described our approach to identify preterm infants at risk for sodium depletion and provide sodium supplementation based on urine sodium concentrations.[13] In sodium deficit states, urine sodium excretion falls too low levels, as renal tubular sodium transporters are activated to the extent possible by neurohumoral and tubuloglomerular mechanisms. Recognizing that there is no consensus regarding the interpretation of urine sodium concentration values, studies in populations of infants with ileostomies and cystic fibrosis demonstrated that sodium supplementation to maintain urine sodium concentrations above certain cut-off values, consistent with a sodium-replete state, is associated with improved weight gain. Using conservative estimates of expected urine sodium concentrations in preterm infants and attempting to account for the immaturity of renal sodium reabsorption mechanisms, we developed an algorithm which to drive sodium supplementation in preterm infants 25–29 weeks gestation (Table 1). Urine sodium concentrations were measured every 2 weeks, beginning at 2 weeks of age, (Fig. 1) and provided sodium supplementation based upon the algorithm until 8 weeks of postnatal age. We then compared the growth of the first 40 infants cared for by this protocol to a recent historical cohort. Sodium intake was on average 1.5–2.0 mEq/kg/d greater in the contemporary cohort (algorithm group) compared to the historical cohort, with 75% of the infants receiving supplementation based upon a low urine sodium concentration. Despite similar caloric, protein, lipid, and fluid intakes between cohorts, infants cared for using the algorithm demonstrated significantly improved growth between 2 and 8 weeks of postnatal age. We are now undertaking a randomized trial using the algorithm to determine its utility and validity in the care of preterm infants (ClinicalTrials.gov NCT03889197). While spot urine sodium concentrations cannot and should not replace more prolonged sampling of urine to measure urine sodium losses, they may be of particular use in infants who are failing to achieve growth goals despite the provision of adequate calories and protein. Additionally, the concomitant administration of drugs that promote natriuresis, such as diuretics, confounds the interpretation of urine sodium values.



Figs 1A and B:. Estimates of urine sodium (Na) concentration and daily urine sodium losses in sodium replete preterm infants based upon literature. Please note that the American Academy of Pediatrics currently recommends sodium intake of 3–5 mmol/kg/d for preterm infants during stable growth phase

Source: Adapted from Segar DE et al. Am J Perinat 2018

The importance of sodium homeostasis in optimizing additional outcomes of preterm infants has been highlighted by other investigators. Both hyper- and hyponatremia in the first week of postnatal life have been associated with increased mortality, risk of intraventricular hemorrhage, and neurodevelopmental impairment,[32–35] Interestingly, children born prematurely and supplemented to sodium intakes of 4–5 mEq/kg/d for days 4–14 of postnatal life had significantly improved neurodevelopmental performance (motor function, performance IQ, the general memory index) at 10–13 years of age compared to infants receiving 1–1.5 mEq/kg/d.[36] Whether the potential effects of sodium on neurocognitive development and brain growth are separate from those on somatic growth is not known. In a mouse model of sodium depletion, we identified that male mice exhibited early-life dietary sodium-dependent improvement in spatial learning and memory, though somatic growth was not different between the two sodium intakes (0.15 vs 0.30% sodium diet).[27] Large studies, we detail sodium intakes and balance, will be needed to address this issue. Late-onset hyponatremia (beyond 14 days of age), which may result from fluid overload or, more likely at this age from total body sodium depletion, has been associated with increased risk of hearing loss, bronchopulmonary dysplasia, and neuromotor and neurocognitive impairment.[37,38]

An area requiring further research is the potential role sodium homeostasis may play in immune function and the risk of infection in preterm infants. In the previously discussed study by Isemann et al., infants randomized to receive the sodium supplementation had significantly lower rates of necrotizing enterocolitis and late-onset sepsis.[31] Over the past decade, there has been emerging evidence regarding total body sodium concentration, including tissue sodium stores, on innate and adaptive immune responses.[39–41] While the majority of work has focused on the effect of high salt intake, Evans et al. identified that adult patients with salt-losing tubulopathies display impaired interleukin-17 responses which link T cell activation to neutrophil mobilization and activation.[42]

Also unknown is the impact of concurrent morbidities, such as bronchopulmonary dysplasia, on kidney function, kidney sodium handling, and ultimately sodium homeostasis. In addition to the state of systemic inflammation, many of the therapeutics used to treat these infants, including diuretics and corticosteroids may impact renal handling of sodium and negatively impact sodium homeostasis and growth. Along these lines, Tan et al. recently reported in infants <28 weeks gestation administration of hydrochlorothiazide and spironolactone for evolving or established bronchopulmonary dysplasia was associated with significant slowing of weight gain.[43]

Conclusion and Clinical Significance

As highlighted in the above text, there is a general lack of understanding of the sodium requirements of the preterm infant. Renal sodium handling in the newborn is inherently related to the stage of kidney development and as such the sodium needs differ based on gestational and postnatal age, as well as confounding medical and surgical conditions. Additionally, the requirements for optimal growth may differ from the requirements to achieve neurodevelopmental outcomes. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Committee of Nutrition recently published new recommendations for enteral nutrient intake in preterm infants, including for sodium.[44] Recognizing the high urine sodium losses that may be present in preterm infants, the Committee now states: "A Na intake of 3–8 mmoL/kg/d is recommended. The upper range of Na intake is slightly higher than in previous recommendations and should be considered in infants receiving high energy and protein intakes or with important sodium loss." These recommendations differ from those of the American Academy of Pediatrics (3–5 mEq/kg/d) which have remained similar for almost 40 years and fail reflect to needs of the extremely preterm infants.[45] We provide recommendations on the range of sodium intakes we believe, based upon currently available data, that meet the sodium requirements of most preterm infants needed to avoid growth failure associated with sodium deficiency. These recommendations are similar to the recommendations of others who have reviewed this topic.[46] We are optimistic that current and future studies will yield a better understanding of the sodium requirements necessary to achieve the full growth potential of preterm infants and develop approaches

to identify infants with sodium deficits who would benefit from additional sodium supplementation.

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REVIEW ARTICLE

Importance of Neuroimaging in Infants with Microcephaly

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Abstract

Microcephaly is diagnosed in infants and children with a head circumference (HC) 2 standard deviations less than average, accounting for age and gender. There is not a standard method of diagnosis, as growth charts vary by country and methodology used. The most popular method of diagnosis is the use of a tape to measure a child's head. There are various conundrums that affect diagnoses: volume of the brain, deformities in skull shape that affect size measurements, and the etiology of microcephaly. The size of the skull is not the most important factor in diagnosing microcephaly, but rather the volume of the brain. Finally, a distinction between primary and secondary microcephaly must be made; primary microcephaly develops prenatally, and secondary microcephaly develops postnatally. The effects of primary microcephaly are generally more severe, but through imaging, it can be detected before birth. This article analyzes various conditions in which neuroimaging can add considerable information to current methods of clinical evaluation. There is a clear need for a multifaceted approach.

Keywords: Aminoacylase-2, Apert, Brain volume, Brain volume loss, Canavan's disease, Cavum septum pellucidum, child abuse, Crouzon, cytomegalovirus, *Ex vacuo* enlargement of ventricles, Head circumference, Meckel-Gruber syndrome, Melting brain, near-drowning, Neuroimaging, Skull deformities, Thalami, TORCH, *Toxoplasma gondii*, Trisomy 13, Trisomy 18, Trisomy 21, Twin-to-twin transfusion syndrome, Vein of Galen aneurysmal malformation, Zika virus, Zika.

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Key Points

- Microcephaly is diagnosed in infants and children with a head circumference (HC) 2 standard deviations less than average, accounting for age and gender.
- There are various conundrums that affect diagnoses: volume of the brain, deformities in skull shape that affect size measurements, and the etiology of microcephaly.
- A distinction between primary and secondary microcephaly must be made; primary microcephaly develops prenatally, and secondary microcephaly develops postnatally.
- This article analyzes various conditions in which neuroimaging can add considerable information to current methods of clinical evaluation. There is a need for a multifaceted approach.

Introduction

Microcephaly in infants and children is defined as a head circumference (HC) 2 standard deviations (SD) below the mean compared with age and gender-matched healthy population.[1–5] Severe microcephaly refers to a HC more than 3 SD below the mean, whereas proportional microcephaly is characterized by HC, height, and weight scalars that are simultaneously 2–3 SD below the mean.[6–10] It is generally acknowledged that HC is correlated to overall brain volume; however, there are many confounding factors that may offer alternatives to this claim.[6,11–14] The HC measures the size of the skull which may be normal, while the brain volume is too small for age.[12,15,16] For example, global brain volume loss with co-existing *ex vacuo* ventriculomegaly may be accompanied by a normal skull size.[9,17–20] An additional limiting factor using the HC as an indicator of microcephaly is that currently no global standards or guidelines exist on using HC growth charts.[21–23] Depending on the included normal population, various growth charts exist.[24–28] As such, determining microcephaly requires a careful selection of the correct growth charts.[25,29,30]

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Microcephaly can be primary, related to a genetic or unknown cause, or secondary, which is obviously related to another illness.[2,3,31–34] Furthermore, the evolution of the HC over time should also be considered.[14,35] Neuroimaging plays a vital role in the diagnostic workup of suspected microcephaly.[36–38] CT and MRI allow qualitative and quantitative evaluation of the brain.[39–41] Combining the data of the HC and neuroimaging findings is essential for adequate diagnosis.[42–43] Neuroimaging should assist to differentiate between microcephaly secondary to a brain malformation, disruption or destruction.[43–45] The goal of this paper

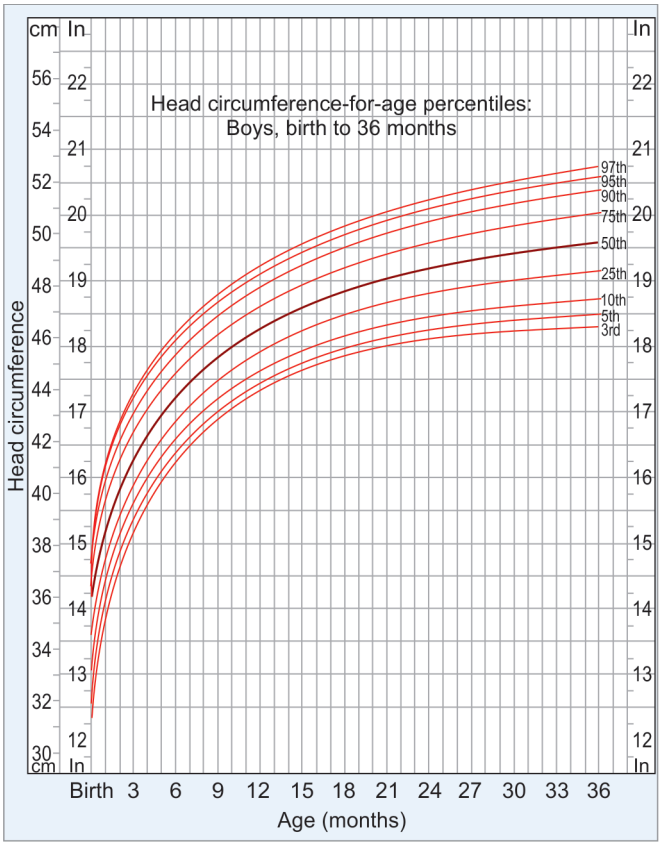


Fig. 1: CDC head circumference chart for boys from age 0 to 36 months

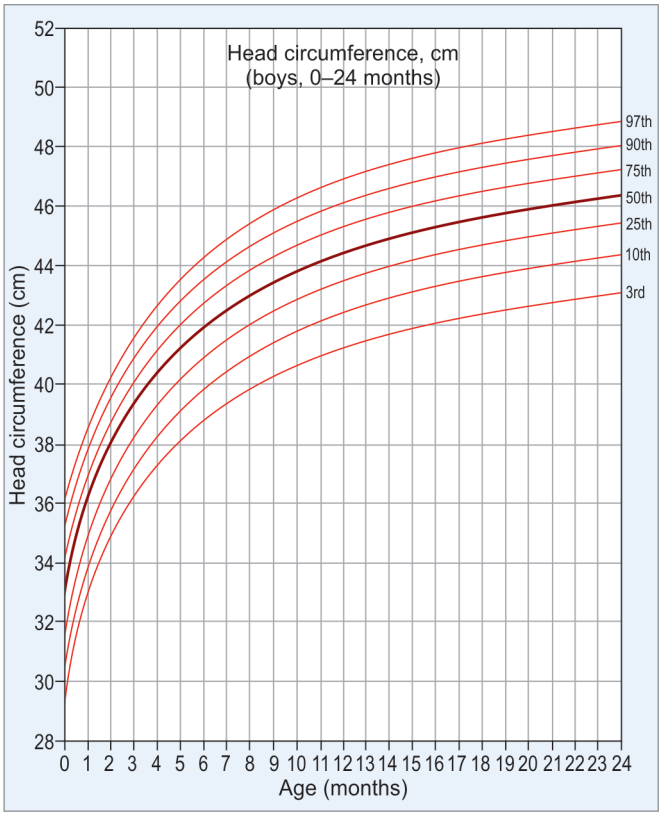


Fig. 2: Brazilian growth chart for boys aged 0–24 months[79]

is to outline the basic aspects of diagnosing microcephaly as well as various anatomical imaging findings that may result in a normal HC measurement while the brain is overall too small in volume. Every (neuro-) radiologist should be familiar with these pitfalls.

Evaluation

Primary microcephaly is usually noticeable at birth, indicating an onset *in utero*. In contrast, secondary microcephaly is acquired postnatally.[7,36] There are many etiologies for both causes of microcephaly, which are addressed in the *Etiology* section of this review.

Prenatally, HC is typically measured with ultrasound studies.[46–52] Using the axial image of the brain at the level of thalami and cavum septum pellucidum, a circular or ovoid curser is placed around the skull to mimic postnatal HC measurement techniques.[53–55] Bi-parietal diameter, as well as other biomarkers, such as the femur length and abdominal circumference, should also be measured to differentiate between an isolated microcephaly versus a proportional microcephaly due to intrauterine growth retardation.[56–61]

Postnatally, HC is first evaluated clinically through cross-sectional and longitudinal measurements.[6,62] The measuring tape is wrapped around the widest possible circumference of the child's head in an axial plane above the eyebrow and above the ears including the most prominent part of the back of the head.[62] Typically, the measurement is done three times and the largest measurement is selected.[63] Temporal evolution of HC is critical and serial HC measurements can be useful if there are concerns such as progressing neurologic deficits.[35,64,65] Infants with microcephaly often present with developmental and intellectual disability, epilepsy, cerebral palsy, language delay, strabismus.[66–68] However, microcephaly may be just one manifestation of a multisystem disorder; cardiac anomalies, renal, urinary tract, and skeletal anomalies may accompany microcephaly.[6,69–74] Consequently, microcephaly requires a full diagnostic workup to exclude additional organ involvement, or multisystem syndromes.

Imaging-related Conundrums

Correct and relevant evaluation of microcephaly can be more challenging than initial impressions. Several misleading conundrums or pitfalls exist of which the (neuro-) radiologist should be aware. The very first conundrum is that there are variations between country-specific growth charts, possibly due to different cultural, socioeconomic or health standards impacting growth or the average height and weight of various age groups in a certain country.[21,75–78] The large ranges of "normal" growth parameters (Figs 1 and 2) may result in an infant being diagnosed to have microcephaly in one, but not in a neighboring country.[8]

A second conundrum is that the size/volume of the brain is much more important than the size of the skull, but external measurements only measure the size/circumference of the skull.[80,81] Imaging in important because even though the HC usually correlates well with the brain volume, there are situations in which slower brain growth leaves "extra space" within the skull.[80]

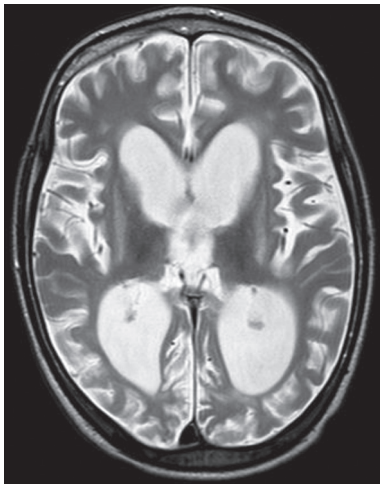


Fig. 3: Axial T2-weighted magnetic resonance imaging showing follow-up changes in an infant who suffered a hypoxic injury. Visible reduction of brain volume, despite the widened sulci, thickened skull, large mastoids, and normal head circumference

Acquired pediatric brain volume loss following accidents such as near-drowning may present with a normal HC with *ex vacuo* enlargement of the ventricles, subarachnoid spaces, and brain sulci.[82] These infants may show widened diploic spaces of the skull on follow-up. Some may also show progressive enlargement of the paranasal sinuses including that of the mastoid air-cell complex, reactive to the brain volume loss.[83] The severity of these manifestations might vary based on age, gender, and ethnicity (Fig. 3). Neuroimaging is essential to identify these pitfalls of a normal HC measurement despite global brain volume loss.[84] Serial imaging and HC measurements beyond the time of an apparent acute injury will increase diagnostic accuracy.[85] Correlation with the clinical history is equally important. An infant with a high-grade hydrocephalus secondary to aqueductal stenosis may initially present with an enlarged HC.[86] After ventriculo-peritoneal shunting, the ventricles may decompress, typically resulting in a decreasing or normalizing HC.[87] The measured HC may however underestimate possible brain volume loss. Long-standing hydrocephalus may impair normal brain development and/or result in irreversible brain injury.[88] The HC may pseudonormalize, or in some cases, the HC remains unchanged/increased due to reactive skull thickening.[89,90]

An additional conundrum seen in many syndromes/systemic diseases is in deformities that alter the shape of the skull, resulting in skewed HC measurements and overdiagnosis of microcephaly.[7] Various syndromic cranio-synostoses, such as Apert or Crouzon syndrome present with a significant skull deformity due to premature closure of skull sutures.[91,92] In these infants, clinical assessment may need to be supported by appropriate neuroimaging.[93,94]

A final conundrum to be recognized is that the overall volume of the brain may not always correlate with neurocognitive and senso-motor functionality.[95,96] For instance, in Canavan's disease, a rare and fatal autosomal recessive degenerative CNS disorder caused by deficiency of aminoacylase-2, infants and young children typically present with an enlarged HC due to a significant increased overall brain volume.[97] However, a few cases might present with microcephaly.[96] Most patients present with intellectual disability, loss of previously acquired motor skills, feeding difficulties, hypotonia, spasticity, paralysis, blindness, and seizures.[97]

Etiology-related Conundrum: Microcephaly due to Malformation, Disruption/Deformation, or Destruction

Primary microcephaly can be seen in the setting of a prenatal brain malformation, brain disruption/deformation, or brain destruction. Familiarity with these three different etiologies is important for correct diagnosis, determining treatment options, predicting outcome, and counseling of the parents and families including recurrence risk for future pregnancies.

Primary, congenital brain malformation refers to an anomalous or abnormal brain development which may be an isolated occurrence such as agenesis of the corpus callosum or secondary to a chromosomal disorder as in Joubert syndrome. Such findings could also be one of the manifestations in a multisystem condition such as Aicardi syndrome. Brain disruption/deformation sequence refers to a process where exposure to toxin(s) as in intrauterine alcohol exposure or in inborn error(s) of metabolism, infections, such as ToRCH (toxoplasmosis, others, rubella, cytomegalovirus (CMV), and herpes simplex, and now including Zika virus infections), or an ischemic/hemorrhagic (thrombo-embolic) event, interferes with the normal brain development. The timing of the event is often more impactful than the intrinsic nature of the injury; the earlier the event occurs during gestation, the more severe might be the resultant brain disruption/deformation sequence at delivery (Fig. 4). In contrast to the "programmed" brain malformation, in the setting of a brain disruption/deformation sequence the brain had the potential to be normally developed. However, many developmental abnormalities may arise in altered neuronal migration, cortical organization, sulcation/gyration, and myelination. In these infants, brain malformations are usually evident at birth. Brain destruction refers to a process where initially normally developed brain is injured/destructed due to an acute event like a focal hemorrhage or ischemia. Congenital microcephaly may be seen in all the settings of brain malformation, brain disruption/deformation sequences, as well as in brain destruction. It is essential for physicians to be familiar with these different qualities and etiologies of microcephaly.

The (neuro-)radiologist should recognize and describe the additional findings that may be seen in children with microcephaly. Focal or diffuse migrational abnormalities, ventriculomegaly, cerebellar dysplasia/hypoplasia, abnormal or delayed myelination, white and gray matter calcifications and white matter gliosis may exist. The combination of findings assists narrowing down the differential diagnosis.

There are many causes of microcephaly. Primary (genetic) microcephaly is believed to result from early exhaustion of the neuronal precursors or accelerated apoptosis.[1] Imaging of the brains of those with primary microcephaly typically show a simplified gyral pattern, shallow sulci, and/or delayed, or impaired myelination. The cortical ribbon is typically of normal thickness with a normal internal laminar organization, the corpus callosum may be thin, and the brain may be hypoplastic but is typically completely formed. Genetic syndromes, including chromosomal abnormalities, are a common etiology of microcephaly. This category includes trisomy 13, 18, and 21. There is often a more severe prognosis in those who develop microcephaly due to a chromosomal abnormality.[36]

Etiologies of Congenital Microcephaly

Congenital infectious microcephaly is often viral (cytomegalovirus, CMV), whereas postnatal microcephaly is more frequently due to

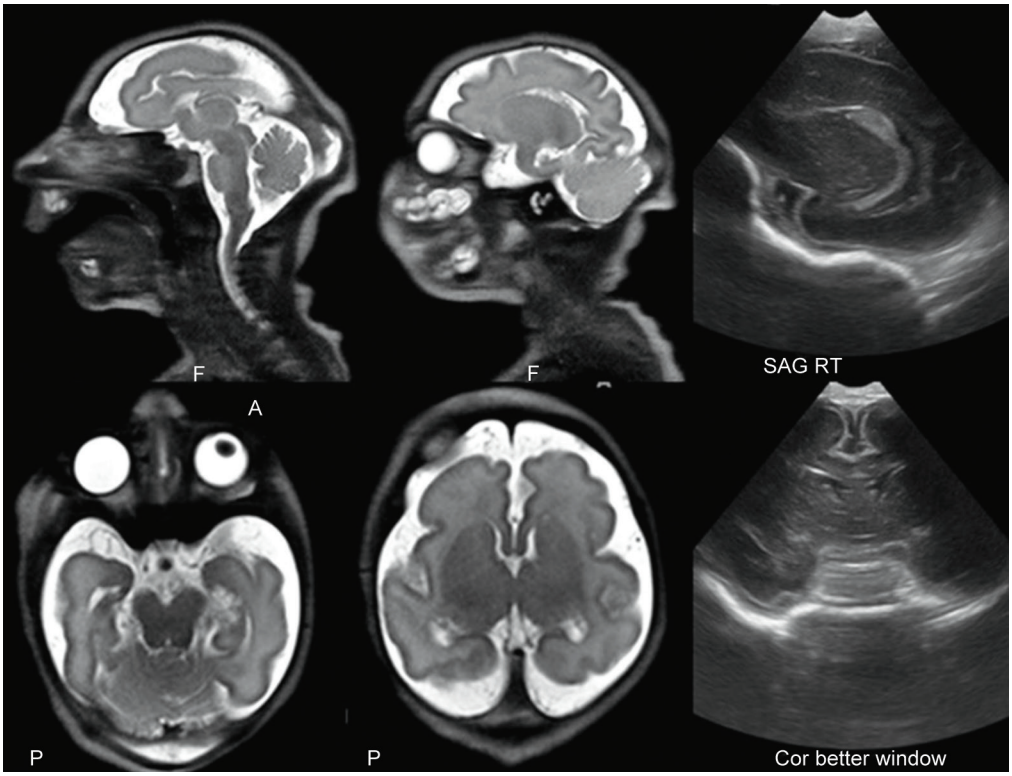


Fig. 4: Primary microcephaly patient shows a simplified gyral pattern

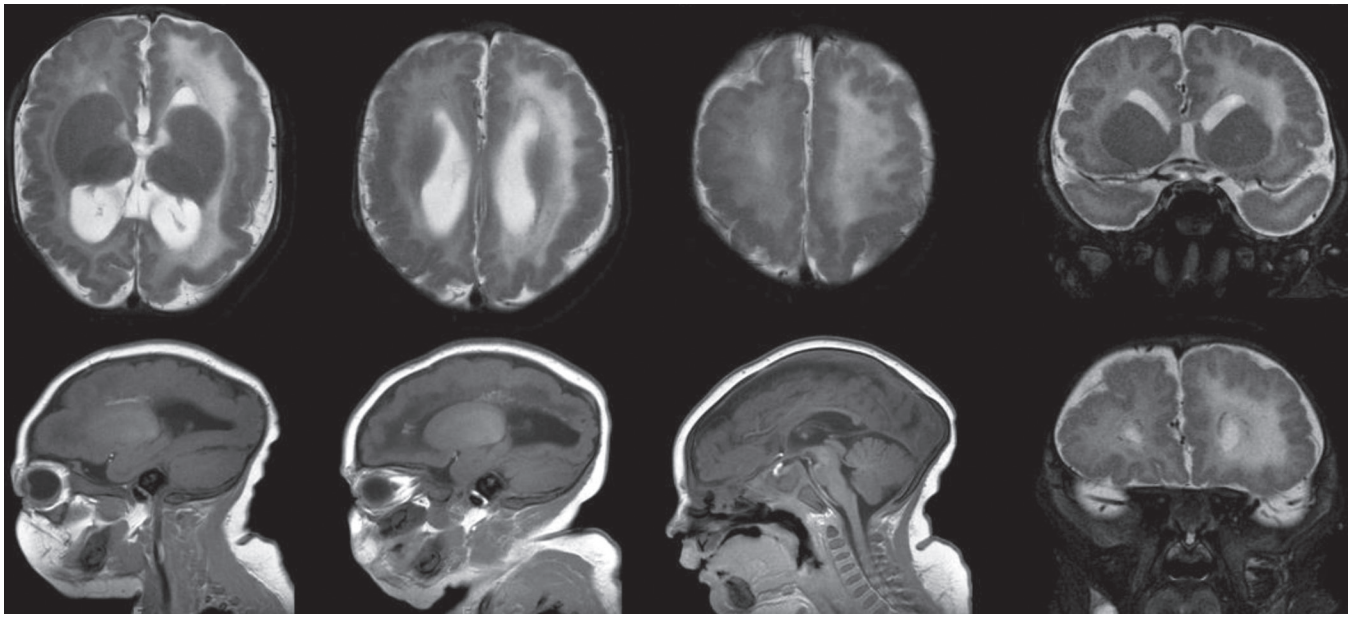


Fig. 5: Microcephaly caused by an infection during early development

a bacterial infection (Fig. 5). Cytomegalovirus infections occurring during early gestation result in white matter loss, ventriculomegaly, cortical anomalies, and microcephaly. Cytomegalovirus infections seen during later pregnancy cause microcephaly less frequently but may still cause many of the aforementioned anomalies. Microcephaly is infrequent in infants who acquire CMV infections during later pregnancy, but many infants may still develop ventriculomegaly. Cytomegalovirus has an affinity for the germinal matrix; intrauterine infections are associated with cortical abnormalities and periventricular calcification. Many patients also show acute and chronic vasculitis, which may cause brain injury due to ischemia.

Intrauterine infections or exposure to teratogens are frequently associated with adverse prognosis. Many maternal diseases have been identified as important causes of microcephaly. Neurotropic infectious agents, such as Zika and TORCH viruses, CMV, rubella,

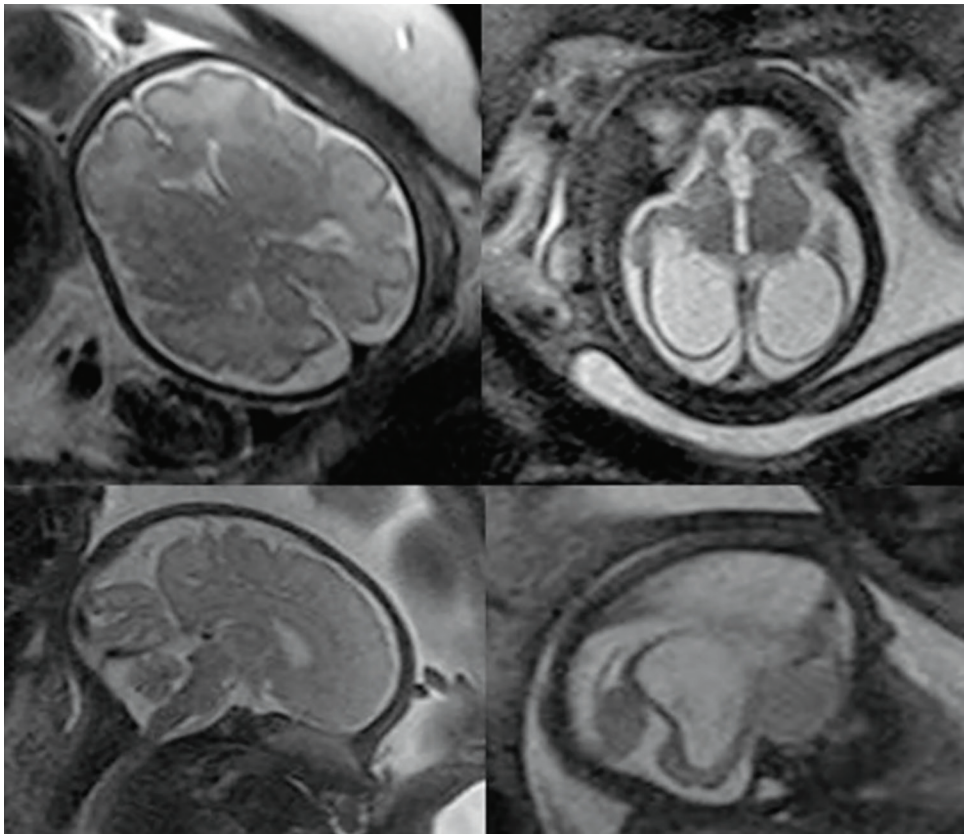


Fig. 6: TTTS twins. The smaller twin on the right has limited white matter and an altered gyral pattern

and *Toxoplasma gondii,* have all been linked with congenital microcephaly. In mothers with phenylketonuria who have high serum levels of phenylalanine, the amino acid may be transmitted to the fetus and at high levels, may act as a teratogen. In other cases, maternal infections may be vertically transmitted to the fetus and cause neural tissue destruction that progresses to calcification.[98] Vertical transmission of the Zika virus can result in global white matter volume loss, a matching small-sized skull, and cortical or central gray matter calcifications. In these fetuses who are infected *in utero*, there may be evidence of overlapping/simultaneous disruptive and destructive processes. These neurotropic infections result in extensive damage in the fetal central nervous system (CNS), particularly if the infection occurs during the first or second trimester. There may also be disruption of subsequent brain development.

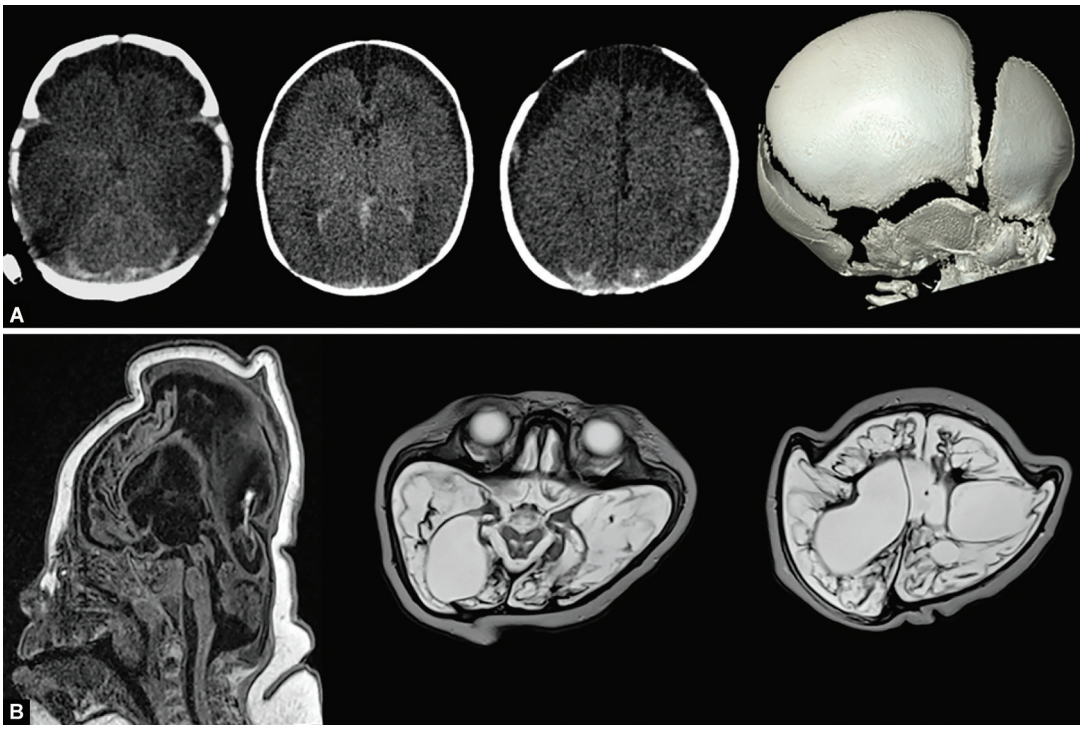
Microcephaly may also be seen in other conditions. For instance, infants who developed twin-to-twin transfusion syndrome (TTTS) *in utero* can show severe microcephaly (Fig. 6).[99] In TTTS, identical twins who shared a placenta (monochorionic-diamniotic pregnancy) may develop injuries due to multiple placental arterio-arterial, arteriovenous, and veno-venous connections.[100,101] These connections disrupt the normal balance of fetal perfusion and blood volumes. In symptomatic TTTS, a "donor" twin shunts/pumps blood to the other "recipient" twin.[102] Due to the excessive blood volume, the recipient twin may develop progressive heart failure while the donor twin remains small for gestational age.[103] Progressive brain injury may result in severe destructive microcephaly.[104] In other infants, microcephaly may also result from progressive heart failure in fetuses with a Vein of Galen aneurysmal malformation (VGAM).[105] The combination of fetal heart failure, systemic and cerebral venous congestion, steal phenomena, and hydrocephalus results in progressive white and gray matter injury which explains the microcephaly. This process is also known as "melting brain."[106] Placental insufficiency, either due to a too small placenta, chorioamnionitis, placental infarctions, or placenta dehiscence with subsequent fetal hypoperfusion or thromboembolic processes are also linked to fetal and neonatal microcephaly.[107–110]

Infants who develop perinatal hypoxic-ischemic injury usually do not have microcephaly in the perinatal period, but they may show progressive brain volume loss during follow-up.[111,112] Similarly, infants who have had to suffer from diffuse brain injury related to child abuse (Fig. 7), near-drowning, trauma, or inborn errors of metabolism with accumulating neurotoxins may develop microcephaly due to arrested brain growth within the first few years.[68,113–116]

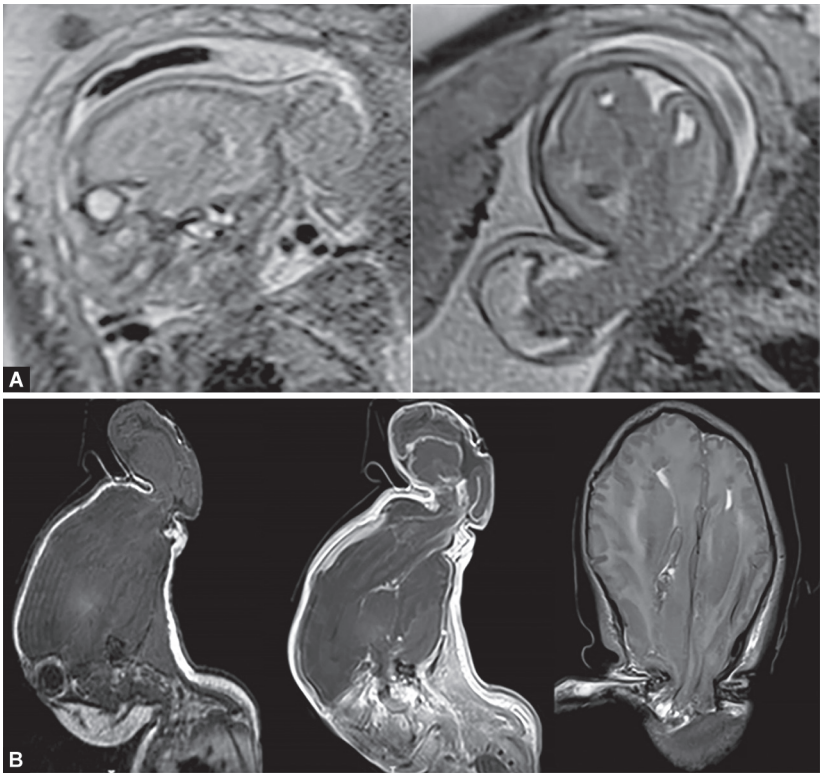
Finally, congenital meningoencephaloceles can also be associated with microcephaly.[117] These are embryonic development abnormalities, characterized by a sac-like protrusion of the brain, meninges, and other intracranial structures through the skull. Nearly 75% of encephaloceles are occipital[118] (Fig. 8). These malformations can be seen as isolated or be seen as a part of multisystem conditions, such as the Meckel-Gruber syndrome.[119–121]

Conclusion

Microcephaly refers to a HC measuring less than 2 SD below average and may be secondary to multiple etiologies, including malformation, disruption/deformation sequence, destruction,



Figs 7A and B: (A) Axial CT including a 3D reconstruction of the skull of a neonate that was a victim of severe child abuse. A global hypodensity of both cerebral hemispheres due to severe brain edema is noted as well as mildly hyperdense blood along the tentorium cerebelli in the subarachnoid space along both occipital lobes as well as within the lateral ventricles. The sutures are widened secondary to global edema; (B) Sagittal T1-weighted and axial T2-weighted MRI of the same child 7 months later shows high-grade, chronic, secondary/destructive diffuse brain volume loss with resultant microcephaly, and partially overriding sutures



Figs 8A and B: (A) Sagittal and axial T2-weighted fetal MRI of a fetus with severe microcephaly secondary to a large malformative occipital meningoencephalocele; (B) Matching sagittal T1-weighted and axial T2-weighted postnatal MRI of the same fetus confirms the extensive occipital meningoencephalocele and microcephaly

and idiopathic forms of microcephaly. Microcephaly may become apparent during a physical exam and the first step is to get a good/reliable HC measurement and then follow-up measurements for temporal evolution. HC has typically been recognized as an indirect marker of brain size. However, a normal HC does not exclude microcephaly. Neuroimaging is essential for correct diagnosis and may reveal anatomical findings that are causative of normal HC measurements. Measuring the HC is not sufficient and may underestimate the degree of microcephaly due to the various conundrums discussed in this paper. The size of the brain is much more important than the size of the skull. There are multiple factors that must be considered in the diagnosis of microcephaly, including but not limited to, primary vs secondary, congenital vs acquired, malformation vs disruption vs destruction. The differential diagnosis list is broad and there are multiple etiologies. Lastly, a multidisciplinary approach is the key to timely diagnosis and appropriate management.

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REVIEW ARTICLE

Neurological Manifestations of Perinatal Dengue

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Abstract

Dengue viruses (DENVs) are single-stranded RNA viruses belonging to the family Flaviviridae. There are four distinct antigenically related serotypes, DENVs types 1, 2, 3, and 4. These are all mosquito-borne human pathogens. Congenital dengue disease occurs when there is mother-to-fetus transmission of the virus and should be suspected in endemic regions in neonates presenting with fever, maculopapular rash, and thrombocytopenia. Although most of the infected infants remain asymptomatic, some can develop clinical manifestations such as sepsis-like illness, gastric bleeding, circulatory failure, and death. Neurological manifestations include intracerebral hemorrhages, neurological malformations, and acute focal/disseminated encephalitis/encephalomyelitis. Dengue NS1Ag, a highly conserved glycoprotein, can help the detection of cases in the viremic stage. We do not have proven specific therapies yet; management is largely supportive and is focused on close monitoring and maintaining adequate intravascular volume.

Keywords: Antibody-dependent enhancement, Congenital dengue, Dengue encephalitis, IgM:IgG ratio, Neonate, Neurotropism, NS1Ag, CYD-TDV (Dengvaxia), TAK-003, Vertical transmission.

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Highlights

- There are four known antigenically related dengue serotypes, named dengue viruses (DENV-1, –2, –3, and –4). The mosquito species *Aedes* (*Ae*.) *aegypti* and *Ae. Albopictus*, are widely distributed in tropical and subtropical areas and serve as vectors for transmission of these viruses.
- Vertical transmission of DENV can be diagnosed if a sample of the umbilical cord, placenta, or newborn peripheral blood tests positive for a DENV diagnostic test in cases with a history of dengue during pregnancy or fever within 10–15 days before delivery in dengue-endemic regions.
- Most infants remain asymptomatic, although some can develop multi-organ system failure. Neurological manifestations can include malformations of the nervous system, acute focal/disseminated encephalitis/encephalomyelitis, and sometimes as a part of the systemic illness, intraventricular hemorrhages.
- Elevated serum levels of interleukin (IL)-6 and IL-8 are associated with neurological involvement and poor outcome.
- Management is largely supportive, and focused on maintaining adequate intravascular volume, breastfeeding, and close monitoring.
- The vaccine CYD-TDV (Dengvaxia) is recommended for persons who are 9–45 years in age, live in endemic areas, and have a confirmed history of dengue infection(s) in the past.

Introduction

Dengue viruses (DENVs) are members of the family Flaviviridae belonging to the genus *Flavivirus*.[1,2] There are 4 distinct antigenically related DENVs, types 1, 2, 3, and 4,[3] and all are mosquito-borne human pathogens.[4] The first case of a pregnant woman with dengue fever was reported in 1948.[5,6]

Viral Structure

Dengue viruses are small spherical viral structures that are typically about 50 nm in diameter and contain a single-stranded RNA genome of positive polarity[7] (Fig. 1). The spherical capsid (shell) is surrounded by an envelope containing numerous copies of M and E proteins.[8] During infections, the DENV envelope *E*-glycoprotein binds viral receptors such as heparan sulfate or lectins in cell surface proteins such as DC-SIGN [Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin, also known as CD209 (Cluster of Differentiation 209)] and the C-type LEctin domain-Containing 5A (CLEC5A).[9–14] Once the viral and cell membranes fuse in acidified endocytic vesicles, the viral RNA enters the cytoplasm and gets translated into a single polyprotein, which is then cleaved to yield 3 structural (capsid, precursor membrane, and envelope) and 7 non-structural proteins (NS1, N2A, N2B, N3, N4A, N4B, and N5).[7] The non-structural proteins play a role in viral replication and modulation

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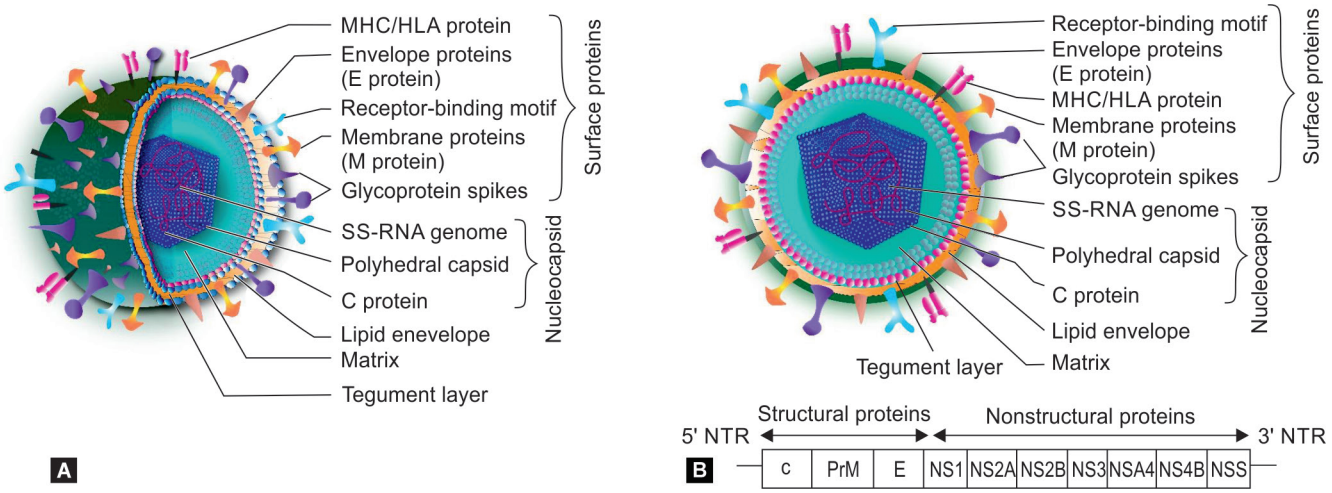


Figs 1A and B: Schematic diagrams showing (A) surface and side dissection and (B) cross-section of the dengue virus

Fig. 2: Global distribution of dengue (regions highlighted with purple color). The disease is frequently seen in Southeast Asia, the Northeastern corner of Australia, sub-Saharan Africa, Eastern Mediterranean regions, Southern Europe, the Middle East, Western Pacific Islands, Mexico, the Southern United States, the Caribbean, and all South American countries except Chile

of the cell antiviral response.[15] NS3 encodes a viral protease which helps in the cleavage of viral proteins.[16] NS5 is an RNA-dependent RNA polymerase, which aids in assembling the replication complex and transcribes the RNA to negative-strand RNA.[17] This strand serves as a template for genomic RNA.[18]

Epidemiology

Dengue virus infection is spread by two Aedes (Ae.) mosquito species, *Ae. aegypti* and *Ae. albopictus*. The DENVs are transmitted in a human-mosquito-human cycle.[6] The incubation period in the mosquito vectors is 8–12 days, after which the virus can be transmitted to humans.[19] In humans, viremia begins after a 4–6-day incubation period and lasts until fever abates.[6,20]

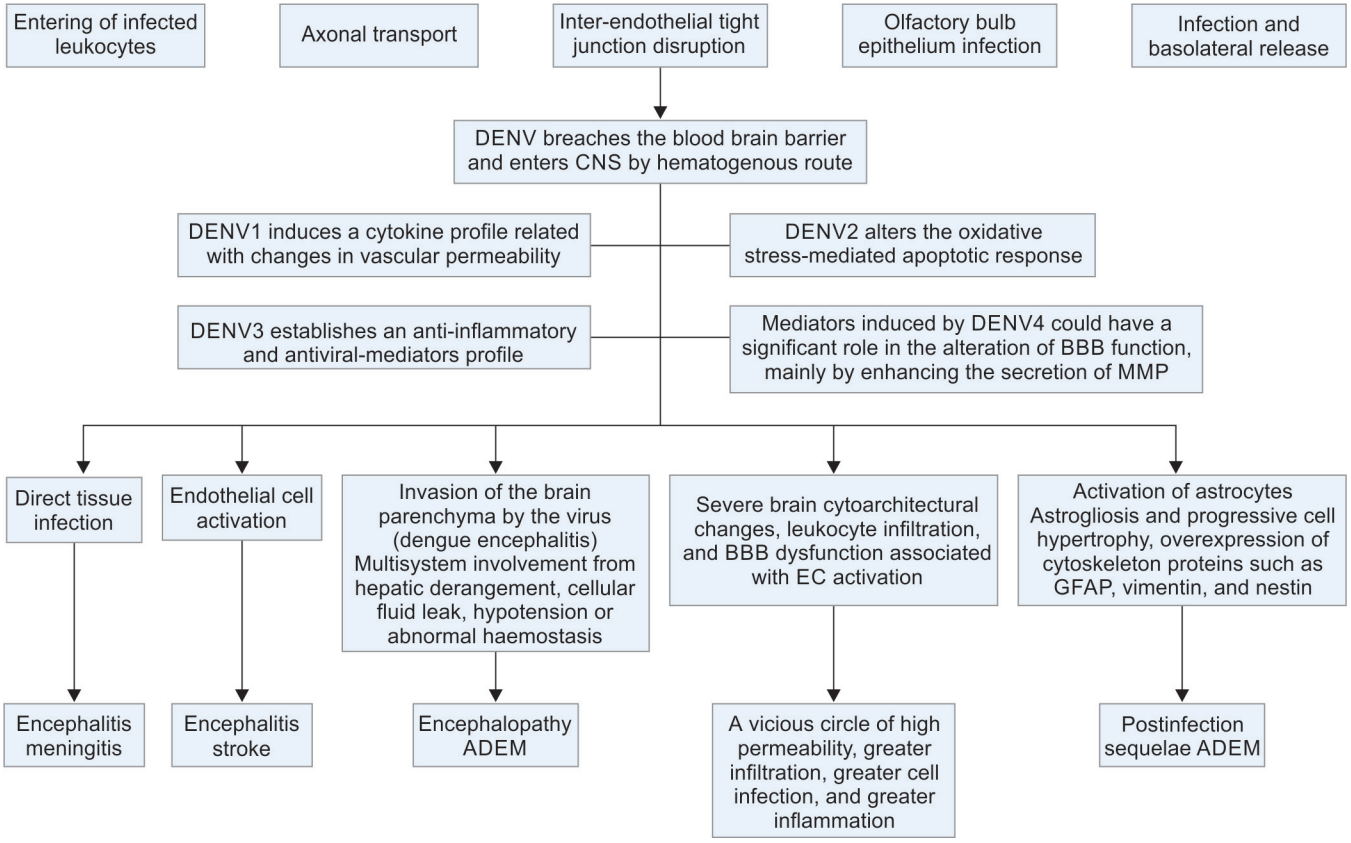
Both *Ae. aegypti* and *Ae. albopictus* are widely distributed in tropical and subtropical areas.[21] *Ae. albopictus* species are more tolerant of cold and have a wider geographic distribution than *Ae. aegypti*.[22,23] *Ae. aegypti* is the most prevalent species in India, Pakistan, and Sri Lanka.[24] A seroprevalence study among children living in India conducted between January 2011 and October 2012 noted 60–80% seropositivity rates.[25,26] The geographical prevalence of these mosquitoes and viruses is depicted in Figure 2.[27–33]

Patterns of Transmission

The DENV transmission follows the following two general patterns, with different implications for disease risk:

- "Epidemic dengue" occurs when a single DENV strain is introduced into a region as an isolated event with a large population of susceptible mosquitoes and human hosts.[34] It can lead to infections among 25–50% of susceptible individuals.[35]
- "Hyperendemic dengue" occurs in areas with a year-round presence of vector mosquitoes, continuous circulation of multiple DENV types, and a large population of susceptible individuals. It leads to repeated epidemics.[36] Children are more susceptible than adults to dengue in hyperendemic regions. Dengue hemorrhagic fever (DHF) is also seen in hyperendemic regions.[37]

Flowchart 1: Pathogenesis of perinatal dengue infections



Factors influencing Transmission

There has been a steady, worldwide increase in DENV infections. The geographic distribution is expanding with population growth and poor urban planning.[38] Global climate change also has an impact on disease transmission with higher global temperatures increasing the range of *Ae. aegypti* and DENVs.[39,40] DENV transmission has also increased with El Niño/Southern Oscillation events.[41,42]

Vertical Transmission

Vertical DENV transmission has been noted in many case series.[43] It should be considered when pregnant women acquire the infection early during pregnancy or at least within 10–15 days prior to delivery. In a prospective study, about 2.5% of women showed a positive immunoglobulin M (IgM) serology. Only 1 (1.6%) of the paired umbilical cord samples was seropositive for dengue although none had evidence of viral RNA by polymerase chain reaction (PCR).

Vertical transmission can increase perinatal morbidity and mortality.[44] DENV is transmitted to the fetus during maternal viremia, but these infected mothers may remain asymptomatic.[45,46] Pregnancy itself has also not been shown to increase the incidence or severity of dengue.[47] Infections in early pregnancy have been noted to cause spontaneous abortions or neural tube alterations in some, but most cases do not show any congenital abnormalities.[48–50] The mode of delivery does not alter the rate of transmission. Newborns with lower weight may be at higher risk of severe dengue. In a prospective study of 2,958 pregnant females,[51] a vertical transmission rate of 18.5–22.7% was reported in a study during an epidemic in French Guiana. Fetal infections seem to be more frequent near term.[46] Breastfeeding has also been reported as a mode of vertical transmission during the postnatal period.[52]

The DENV serotype 2 has been the predominant serotype associated with vertical transmission.[53] This may be explained by the high circulation of DENV serotype 2,[54] or the ability of this serotype to cross or disrupt the placental barrier. Sequential fetal growth monitoring should be undertaken in pregnant women with dengue to screen for fetal growth restriction and stillbirths.[55]

Course of Infection

The course of infection by the DENVs can be subdivided into early events, dissemination, and the immune response and viral clearance:

- Early events refer to the inoculation of DENV into a susceptible host. Dissemination is manifested as viremia, 2–6 days after subcutaneous inoculation, and may last up to 3–6 days.[56]
- Immune response and viral clearance are achieved through innate and adaptive immune responses.[57] Neutralization requires a threshold level of antibodies.[58] Sub-threshold levels may paradoxically increase the uptake of antibody-bound viruses.[59] This phenomenon has been described as antibody-dependent enhancement (ADE) of infection.[46,60]

Primary vs Secondary Infection

Infection with one of the four serotypes of DENV (primary infection) confers long-lasting specific immunity to viruses of that serotype.[50] There might be some, transient immunity to the other serotypes, and subsequent infections can still occur with the other serotype (secondary infection).[61] In these secondary infections,

Figs 3A to D: Clinical manifestations of congenital dengue in neonates. (A and B) Images of infants showing maculopapular rash; (C and D) Images of infants showing microcephaly due to congenital dengue

the concentration of DENV-specific antibodies increases earlier with higher peak titers and lower IgM:IgG ratio, suggestive of an anamnestic response.[62] High levels of DENV-specific antibodies may be seen in later stages of viremia, increasing the formation of immune complexes and activation of complement.[63]

Neurological Manifestations

Flowchart 1 shows the neurological manifestations. The neurological manifestations of congenital infections may result from (A) direct infection of neurological tissues (encephalitis, meningitis, myositis, myelitis, rhabdomyolysis; (B) systemic or metabolic imbalance (encephalopathy, stroke); and (C) early or late postinfection sequelae (transverse myelitis, acute disseminated encephalomyelitis).[64]

Furthermore, DENVs have strong CNS tropism.[65] These viruses enter the CNS *via* the hematogenous route.[66,67] These viruses activate endothelial cells (ECs), breach the blood–brain barrier (BBB), infect neurons, and induce cytoarchitectural changes.[68] These can reach the brain parenchyma: (A) in infected leukocytes; (B) through axonal transport; (C) *via* infection of the olfactory bulb epithelium; (D) by disrupting the inter-endothelial tight junctions; and (E) *via* endothelial infection and basolateral release.[69,70] The latter two mechanisms and viruses carried in infected monocytes may be the most critical routes.[65] To recapitulate, most DENV strains are neurotropic and neurovirulent, able to evade the immune system and invade the brain efficiently through BBB ECs, leading to replication in the brain parenchyma which induces nervous injury.[68,71]

An EC cross-activation following infection involves the soluble vascular cell adhesion molecule (sVCAM-1) and soluble intercellular adhesion molecules (sICAM-1).[72] The infected endothelium secretes immune mediators; DENV1 is known to induce interleukin-6 (IL-6), tumor necrosis factor (TNF), chemokine (C-X-C motif) ligand 1 (CXCL1), CCL2, CCL5, and CCL20.[73] These molecules have been associated with endothelial hyperpermeability and also with an imbalance in the coagulation pathway leading to microhemorrhages. Many neonates with severe infection can develop disseminated intravascular coagulation.[74,75]

In the eyes, the virus enters through the hematogenous route and infects the endothelium, pericytes, and other cells.[76] Pericytes augment the infection by secreting several immune mediators that modify the barrier physiology. In the CNS, the glia are also infected.[77–79] Activated astrocytes show altered function, morphology, and biochemical reactions.[80] These cells begin to secrete proinflammatory molecules such as IL-6, TNF, and interferon-β.[81,82] Strong, continuous stimuli have been associated with astrogliosis and cellular hypertrophy with longer and thicker astrocytic processes;[83] overexpression of cytoskeletal proteins such as the glial fibrillary acidic protein (GFAP), vimentin, and nestin[84] results in glial cell proliferation and scar formation.[85]

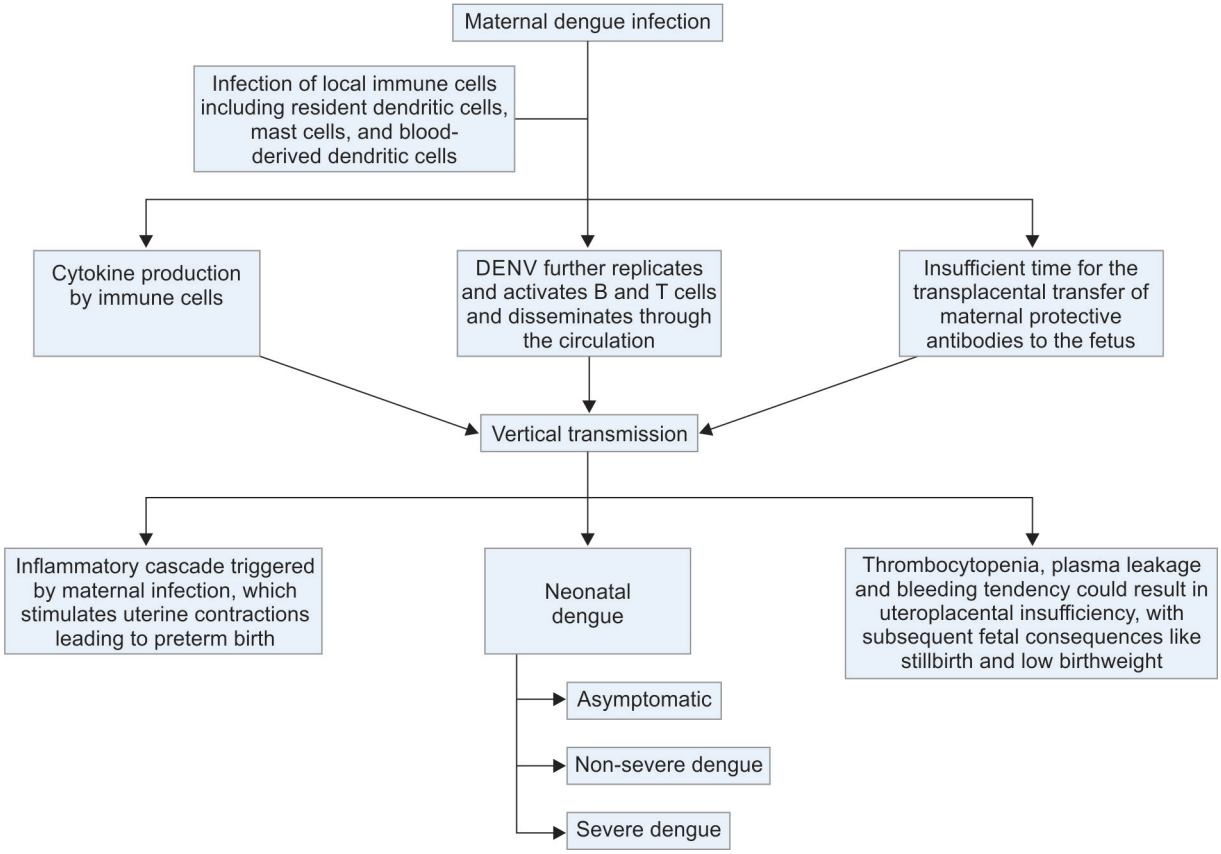
Encephalopathy is a recognized complication of dengue,[86,87] and is usually ascribed to the neurotropism of these viruses and consequent invasion of the brain parenchyma (dengue encephalitis). Multisystem involvement from hepatic derangement, cellular fluid leak, hypotension, and altered hemostasis worsens the illness. In a case–control evaluation of the cytokine response in patients with DENV, elevated levels of IL-6 and IL-8 were associated with severe neurological manifestations and poor outcomes.[88] In an *in vitro* study using BV2 microglial cells,[89] various DENV serotypes induced different responses. DENV1 induced a cytokine profile that altered vascular permeability, whereas DENV2 altered the oxidative stress-mediated apoptotic response.[90] Also, DENV3 established a distinct response with anti-inflammatory and antiviral mediators. DENV4 altered the BBB by inducing matrix metalloproteins.[89]

Clinical Presentations

Congenital dengue occurs when there is insufficient time for the induction/transplacental passage of protective antibodies postmaternal infection.[91] It should be suspected in neonates presenting with fever, maculopapular rash, and thrombocytopenia in endemic regions (Figs 3A and B). Both the mother and baby should be simultaneously evaluated by tests for DENV antigens and serology.[91]

Maternal DENV infections have been shown to increase the incidence of prematurity.[44,57,92] This association can be explained by inflammatory changes triggered by maternal infection, which stimulate uterine contractions (Flowchart 2). There is increased production of pro-inflammatory cytokines such as IL-6, –8, and TNF, which stimulate the uterus, leading to preterm labor.[5,6,20,93] There is conflicting evidence regarding the correlation between the severity of neonatal and maternal dengue, factors affecting vertical transmission, and disease onset.[5,57,94] The longer the time from the onset of maternal fever to giving birth, the sooner the occurrence of fever in the neonate with an incubation period of 5–6 days.[95] Petechiae in neonatal dengue are seen more frequently as compared to older infants and children.[96] Hemoconcentration is not a reliable parameter in neonatal dengue because of an increased red blood cell mass with a higher hematocrit compared to older children and adults.[97,98] Investigations such as chest X-ray, renal and

Flowchart 2: Pathogenesis of neurological manifestations of DENV infections



liver function tests, and ultrasonography of the chest and abdomen may be done as clinically indicated.[99]

Mild-moderate maternal DENV infections have not been clearly associated with intra-uterine growth restriction/low birth weight.[5] However, in severely afflicted cases, hypovolemia resulting from plasma leakage and hemorrhages could result in uteroplacental insufficiency leading to fetal growth restriction and even demise.[100] In a study of 44 pregnancies from India,[55] there were miscarriages in 2 (4.5%), stillbirths in 4 (9%), and neonatal deaths in 2 (4.5%). There was preterm delivery in 15 (34.1%) and the infants were born with low birth weight in 13 (29.5%).[101,102]

Population studies do not show *in utero* DENV infections as consistently increasing the rates of cesarean sections in infected women or as a cause of congenital anomalies in affected neonates.[51,55] In some of these studies, the small number of infants with clearly evident clinical features may have resulted in the lack of statistically significant differences. A small number of infants with early onset *in utero* infections show neurological manifestations such as microcephaly (Figs 3C and D), anencephaly, and hydrocephalus.[60,103] Most of the infants with later-onset infections that likely occurred during the perinatal period, developed encephalopathy and had an uneventful recovery.

Peripartum maternal dengue makes newborns susceptible to complications because most of the transplacentally delivered antibodies lack a protective effect[47] and instead, enhance the entry of virus into the host cells.[104,105] Postnatal dengue infections are usually asymptomatic, although some infants may manifest with undifferentiated fever, upper respiratory tract symptoms, vomiting, and diarrhea. Liver involvement is more frequent in infants compared to older children.[106] The higher frequency of DENV hepatitis can be explained by the tropism of these viruses for liver cells.[107] Many studies show that the diagnosis of neonatal dengue requires a high index of suspicion.[43,44,46,108] Infants with cutaneous manifestations and/or fever frequently show hepatomegaly.[98] It is seen more frequently in epidemic-region countries where pregnant women could get infected near the time of delivery.[5,44,93]

Neonatal dengue can be a difficult diagnosis.[109,21] In one study, 12 out of 32 cases were classified as neonatal sepsis or neonatal immune thrombocytopenia.[110] Neonates who are diagnosed with dengue should be monitored for warning signs of shock or severe hemorrhages. The hemorrhagic manifestations are usually mild and are usually limited to petechiae.[110] Total leukocyte counts can drop during the febrile phase but then normalizes in the critical phase.[110] Some infants may show gall bladder wall thickening.[20,26,49,111–113] Monitoring for complications should continue for 24–48 hours after defervescence.

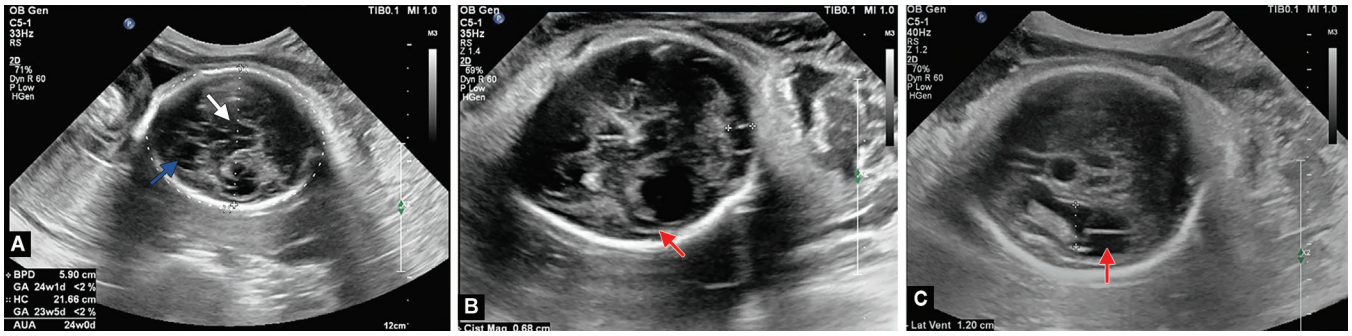
Pregnant women should avoid travel to *Aedes* spp. endemic regions. Post-travel laboratory testing should be reserved for symptomatic patients.[114]

Laboratory Diagnosis

The diagnosis of congenital DENV infections is based on the history of maternal fever and the presence of either dengue nonstructural 1 (NS1) antigen or dengue antibodies.

Enzyme-linked Immunosorbent Assay-based NS1 Antigen Tests

Dengue NS1Ag is a highly conserved glycoprotein, which is produced in both membrane associated and secreted forms and is abundant in the serum of patients during the early stage of dengue



Figs 4A to C: Antenatal scan of a mother at 29 weeks, 3 days gestation. (A) Enlarged extra-axial CSF spaces with ventricular dilatation (white arrow) and cystic changes (blue arrow); (B) Thinning of parenchyma (red arrow); and (C) Ventricular dilatation (red arrow)

infection. It helps in the detection of cases early in the viremic stage.[5,57,94] Other diagnostic options are dengue virus isolation or detection of antibodies.

Serologic Assay for DENV

Enzyme-linked Immunosorbent Assay

It is a simple test based on detecting the dengue-specific IgM antibodies in the test serum by capturing them using antihuman IgM bound to the solid phase.[115] After adding dengue antigen, if anti-dengue IgM is present, it will bind and give a color reaction with the enzyme substrate. Antidengue IgM is detectable by day 5 of the illness.[116] For single serum, enzyme-linked immunosorbent assay (ELISA) IgM titer more than or equal to 1 or an IgG titer above 3 is considered evidence of acute and/or recent DENV infection. An ELISA IgM:IgG ratio of above 1.8:1 is considered a primary infection. Acute DENV infections are typically associated with a 4-fold or higher rise in antibody titers.[117]

Hemagglutination (HI)

Seroconversion or high titers (≥1:2560) are suggestive of recent infection. Moreover, WHO recommended using HI titers of convalescent sera as the criteria to distinguish between primary and secondary infection. The infection is diagnosed as primary if the titer in a week or more after the onset of illness is above 1:1280 or as secondary if antibody titers are more than or equal to 1:1280.[118] Also, HI antibody is used in laboratories and is believed to assess seroprotection; Ig-G ELISA compares well to the HI test.[118]

Dengue Viral Isolation

Tissue Culture

Serum samples are inoculated into tissue culture flasks containing *Ae. albopictus* mosquito cell monolayers.[119] After 90 minutes of adsorption of the inocula on cells at 28°C, cell cultures are incubated for 7 days at 28°C. Cells are harvested for the identification of viruses by indirect immunofluorescence staining. Virus isolation and nucleic acid tests have high specificity but are expensive and labor intensive and are used for early detection in the first week of illness.[120] Virus isolation takes around 7–10 days.

Mosquito Inoculation

Dengue viral isolation has been attempted with laboratory-reared mosquitoes (*Toxoshynchites splendors*) by an intrathoracic inoculation technique.[121] Identification of DENV serotypes is performed by indirect fluorescence antibody staining using serotype-specific monoclonal antibodies.[122]

Reverse Transcription Polymerase Chain Reaction

The reverse transcription polymerase chain reaction (RT-PCR) can be used to detect DENV RNA. Nested RT-PCR, real-time RT-PCR, or nucleic acid sequence-based amplification (NASBA) can be used.

Pauci-symptomatic dengue cases may be underdiagnosed during the neonatal period because DENV infections during pregnancy are not identified.[46]

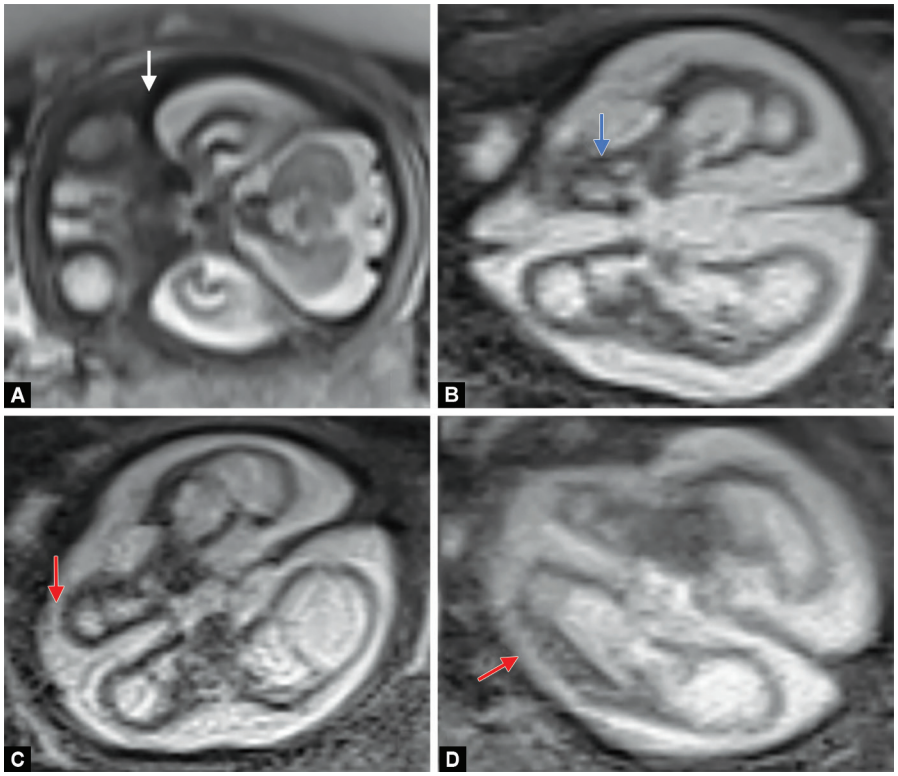
The "diagnosis of vertical transmission" of dengue can be made if a sample of the umbilical cord, placenta, or newborn peripheral blood collected immediately postpartum reveals a positive result in a dengue diagnostic test.[46] Umbilical cord blood and placenta should be tested if there is a history of dengue during pregnancy or fever within 15 days before the term in dengue-endemic regions. Positive results indicate a need for close clinical monitoring of the newborn; peripheral blood samples should be tested if the infant becomes symptomatic.

The "diagnosis of neurological manifestations" may require an ultrasound of the skull or a magnetic resonance imaging (MRI) brain.[123] The cerebrospinal fluid (CSF) studies are required to diagnose dengue encephalitis. Tests for CSF IgM, IgG, and NS1 antigen should be performed if neurological manifestations are present.[124] Carod–Artal et al.[125] defined dengue encephalitis if each of the following criteria were fulfilled: (A) Dengue CNS involvement; (B) Presence of dengue virus RNA, IgM, or NS1 antigen in CSF; and (C) CSF pleocytosis without other neuroinvasive pathogens.

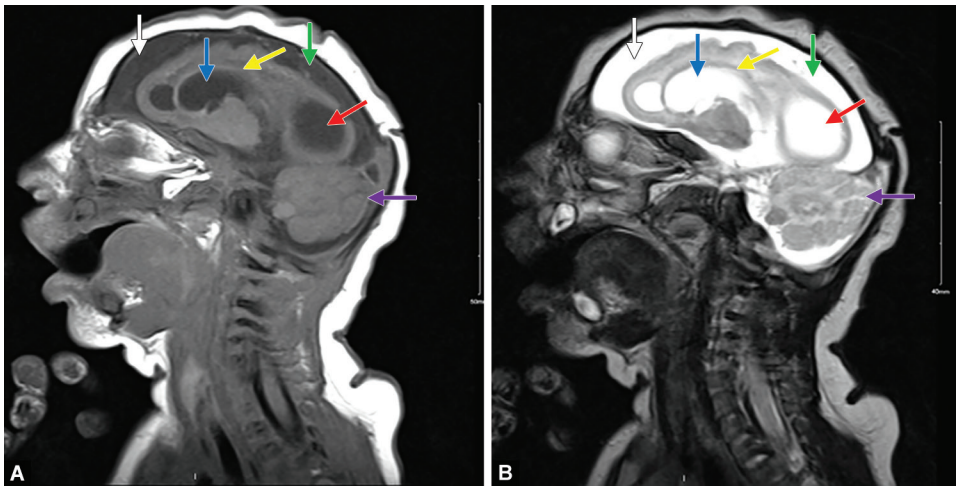
Pleocytosis in CSF may not be seen in 5% of encephalitis cases, especially early in the course of dengue.[126,127] Furthermore, IgM antibodies in CSF have high specificity but low sensitivity; these appear only by the seventh day following infection.[128] Hence, the absence of antibodies does not exclude the neurological manifestations associated with dengue.

In view of these limitations, another definition was suggested for dengue encephalitis: (A) presence of fever; (B) acute signs of cerebral involvement such as altered consciousness or seizures and/or focal neurological signs; (C) reactive IgM dengue antibody, NS1 antigen, or positive dengue PCR on serum and/or CSF; (D) exclusion of other causes of viral encephalitis and encephalopathy.[129] This definition will reduce the number of missed cases of dengue encephalitis. Brain-evoked response auditory (BERA), visual-evoked potential (VEP), and follow-up MRI should be considered. These infants need follow-up for neurocognitive outcomes.[130]

Congenital DENV infections can show notable CNS lesions on imaging (Fig. 4). However, many of these findings are not characteristic of *in utero* infections and can also be seen in infants

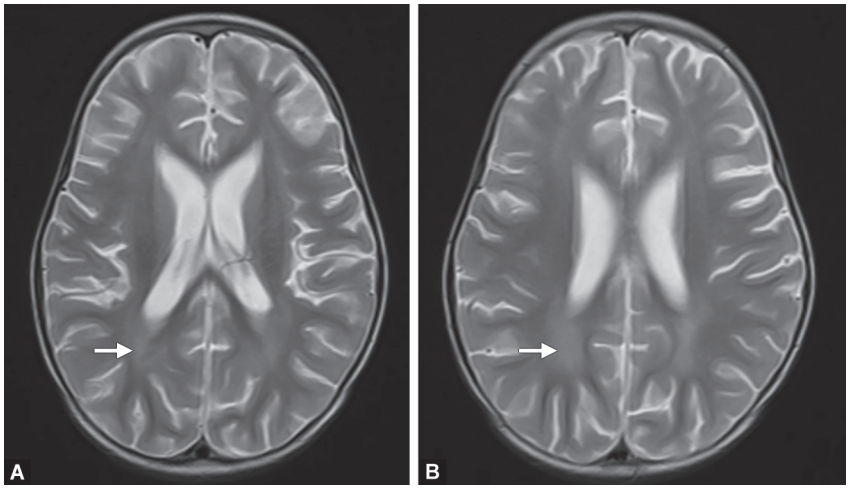


Figs 5A to D: Fetal MRI at 29 weeks, 6 days. (A) Enlarged extra-axial CSF spaces with ventricular dilatation (white arrow); (B) Cystic changes of the bilateral frontal lobes and left parietal area (blue arrow); (C) and (D) Images showing thinning of the parenchyma (red arrows), which resulted in the loss of volume and architecture of the cerebral parenchyma



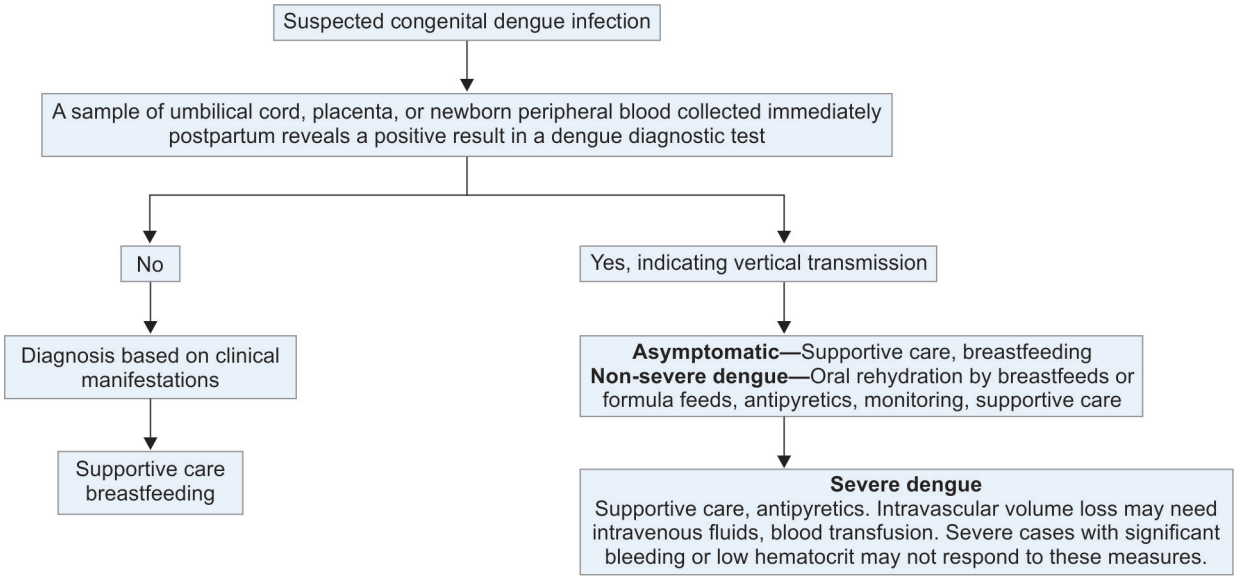
Figs 6A and B: Postnatal MRI on day 8 after birth (A) T1W image; (B) T2W image. Findings show decreased brain parenchymal thickness, especially in the supratentorial lobes with marked simplification of gyral patterns (white arrows), loss of normal fissurization, and operculation. Other findings include ventriculomegaly (blue arrows), cystic changes (red arrows), and altered formation of the cerebral cortex with thinning of the corpus callosum (yellow arrows). Additional features are enlargement of the subarachnoid spaces (green arrows), bilateral open sylvian fissures and cerebellar hypoplasia (purple arrows)

who acquire the virus after birth. In a retrospective study of 36 patients with serologically proven DENV infections with neurological symptoms,[131] The MRI did not show any abnormalities in 11, showed an encephalitic pattern in 12, encephalopathic (seizure related/metabolic) findings in 4, acute disseminated encephalomyelitis (ADEM) in 3, and isolated micro- or macro-hemorrhages in 6. Such a pattern-recognition approach may help in identifying the pathology, differential diagnosis, and in making treatment decisions. In another study,[132] the basal ganglia, thalamus, brainstem, cerebellum, cortical white matter, periventricular white matter, and cortical gray matter were most frequently involved and appeared hyperintense on T2-weighted images, and the fluid-attenuated inversion recovery (FLAIR) protocol. These lesions appeared iso- or hypo-intense on T1-weighted images. "Blooming" micro-hemorrhages were seen



Figs 7A and B: The MRI brain (T2-weighted, two levels) of a 2-year-old child with dengue encephalitis showing periventricular hyperintensities along bilateral lateral ventricles (white arrows). The changes are most prominent along the posterior horns

Flowchart 3: Management of perinatal dengue infections



on susceptibility-weighted MRI (Figs 5 and 6). Children who are infected or manifest at later ages typically show less severe imaging changes (Fig. 7).

Clinical Management

Treatment

No credible anti-DENV therapy is currently available.[133] Management is supportive with close monitoring and is focused on maintaining adequate intravascular volumes (Flowchart 3). In mild cases, oral rehydration by breastfeeds or formula feeds is sufficient. Acetaminophen (maximum 60 mg/kg/day) can be used for the management of fever. Aspirin or nonsteroidal anti-inflammatory agents should be avoided because of the risk of bleeding complications and potentially of Reye's syndrome in infants.

Plasma leakage should be managed with intravascular volume repletion to prevent hypovolemic shock. Infants with established intravascular volume depletion may require intravenous fluids. Blood transfusions may be needed in patients with significant bleeding and anemia, and inadequate response to fluid resuscitation.[134–136] Acidosis, hypoglycemia, and hypocalcemia should be investigated and corrected as needed. Prophylactic platelet transfusion is not recommended.[137] Fresh frozen plasma may be used in cases with coagulopathy with bleeding.

Breastfeeding

The secretion of DENV in human milk is uncertain and likely very rare, even though there are some positive reports.[52] Breastfeeding is encouraged in infants of infected mothers.[138] Human milk contains antiviral antibodies that may provide protection.[139] Neonates may be discharged once afebrile for 24–48 hours, hemodynamically stable with good urine output, and accepting feeds well.

There is no role for corticosteroids,[140–142] intravenous immunoglobulins, pentoxifylline, or activated factor VII.[143–145] Direct viral inhibitors and modifiers of virus-host interactions are under investigation.[146,147] Chloroquine, lovastatin, balapiravir (a polymerase inhibitor), and celgosivir (an α-glucosidase inhibitor)

have not been shown to have any benefit in randomized controlled trials.[148–150]

Outcomes

The DENV infections during pregnancy can increase the overall risk of neurologic anomalies by 50% and of congenital malformations of the brain by 4-fold.[102] The biological mechanism(s) for this teratogenicity are unclear, but there is evidence for the DENV virus crossing the placental and blood–brain barriers[102,151,152] and for its neurotropism.[125,153] The DENV antigen and antibody testing in CSF has procedural inconsistencies, limited availability, and variable sensitivity and specificity.[154] An MRI of the brain may reveal hyperintensities in globus pallidus known as the "double doughnut sign".[155] Dengue encephalitis should be considered as a possibility in an infant with dengue fever with altered sensorium.

Unlike fetal dengue, the outcomes of postnatal infections seem more encouraging but still need further study. One study from Thailand showed normal growth and development in all infants with neonatal dengue at 1-year follow-up.[53] Some neonates can recover even from ADEM following vertically transmitted infections. The mothers had a history of febrile illness before delivery; the infants developed fever, lethargy, poor feeding, and seizures that lasted for up to a week. The MRI scans showed multiple areas of restricted diffusion of the white matter in the frontoparietal and temporal lobes and internal capsules. However, even though the severity of ADEM can vary between patients, many recover over time probably because the immune response differs from that in adults and does not augment tissue damage.[4,151] In one case reported from India, the neonate fulfilled all criteria for dengue encephalitis; there was fever, lethargy, and seizures, positive serology for NS1 antigen, and detectable titers of DENV IgM antibody in serum and CSF. Management includes supportive measures and phenobarbitone.[156] There was gradual recovery without any sequelae.

In neonatal and adult murine models infected by intranasal inoculation, DENV serotype 2 showed brain tropism with encephalitis.[157] After invading the upper respiratory tract mucosa, it likely entered the brain through the olfactory nerve with massive viral replication. There were neurological symptoms.[69] Affected areas showed considerable leukocyte recruitment, but paradoxically, these cells may have increased the severity of encephalitis owing to the Trojan horse effect.[158,159]

Prevention

Approaches for the prevention of DENV infection in endemic areas may include vaccination, mosquito control, and personal protective measures.[160]

Vaccine Development: Infection with one DENV type provides long-term protection against reinfection with that same type and a short-lived cross-protection against the other DENV types.[161]

The vaccine CYD-TDV (Dengvaxia) has been licensed in many countries in Latin America and Southeast Asia. It is a formulation of four chimeric yellow fever 17D-dengue vaccine viruses.[162,163] The vaccine shows 75% efficacy against DENV-3 and DENV-4, 50% for DENV-1 and 35–42% for DENV-2. According to the WHO, the vaccine is protective against severe dengue for individuals with dengue seropositivity at the time of first vaccination. Vaccine efficacy is lower (34–36%) in children 2–5 years of age and in children who do not have detectable dengue-neutralizing antibodies prior to vaccination.[164–166] Two vaccines are in clinical development, the TAK-003 and a tetravalent, live-virus vaccine attenuated by directed mutagenesis with a DENV-2/-4chimeric strain.[109,111,112] TAK-003 is tetravalent vaccine based on an attenuated laboratory-derived DENV-2 virus.[167–170] Further studies are required to evaluate efficacy and safety, especially for DENV-3 and DENV-4.

Mosquito Control: The methods that are most efficacious involve reducing breeding sites and larva control. Seeding water vessels with copepods (these are small crustaceans found in most freshwater and saltwater habitats) that feed on mosquito larvae can eliminate *Ae. aegypti* and dengue transmission. Endosymbiotic control can be achieved by releasing mosquitoes infected with

Table 1: Major structural components of DENVs

Structure	*Available information*
Lipid envelope	The nucleocapsid is surrounded by a lipoprotein envelope derived from the nuclear membrane of the infected host cell.[7]
Glycoproteins	Projecting from the lipid envelope are viral glycoprotein spikes that bind specific host receptors to facilitate virus entry. DENV binds to cells by the major viral envelope (E) glycoprotein, which is critical for infectivity.[9–11]
Receptor binding motifs	Receptor binding motifs are involved in virion attachment to cell surface receptors. DENV infection begins with virus attachment to the target cell by the interaction between viral surface proteins and receptors on the cell surface leading to the internalization of the virus by receptor-mediated endocytosis.[174–176]
Envelope protein	The nucleocapsid is surrounded by a trilaminar lipoprotein envelope containing envelope protein or the "E" glycoprotein.[9–11,177]
Membrane protein	The virus particles have two surface viral proteins: the E (envelope) glycoprotein, which is the major determining antigen and involved in binding and fusion during viral entry, and the M (membrane) protein, a part of the precursor prM, formed during the maturation of the virus. M acts as a secretory protein analogous to the major envelope protein E.[178]
MHC or HLA proteins	Some MHC gene combinations can act synergistically to influence disease expression in previously DENV-exposed individuals.[179]
Spike protein	Projecting from the lipid envelope are viral glycoprotein spikes that bind specific host receptors to facilitate virus entry.[178]
Surface tubules	Either not expressed or relevance unclear fetal/infantile disease
Palisade layer	Either not expressed or relevance unclear fetal/infantile disease

Viral tegument	Either not expressed or relevance unclear fetal/infantile disease
Lateral bodies	Either not expressed or relevance unclear fetal/infantile disease
Capsid	The mature capsid of DENV is a highly basic protein of 12 kDa that forms homodimers in solution and has an affinity for nucleic acids and lipid membranes. It exists as a 100-residue monomer and contains 26 basic amino acids and only 3 acidic residues.[8]
Capsomeres	The proteins that compose the structural unit may form three-dimensional structures known as "capsomeres" that are visible in an electron micrograph.[8]
Core membrane	Either not expressed or relevance unclear fetal/infantile disease.
Protein core	The polyprotein produced by dengue virion is processed into three mature structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[180]
Core fibrils	DEN2 virus-infected apoptotic cells also show bundles of intracellular microfibrils which resemble the contractile structures observed in fibroblasts and some glomerular cells. These structures could be related to the apoptotic process, since, filamentous material, clumping of tonofilaments and MyD88 protein.[181]
Matrix	Either not expressed or relevance unclear fetal/infantile disease
Enzymes	The only known enzymes of DENV are encoded by NS3 and NS5 proteins. The N-terminal domain of NS3 is a protease (with NS2B as a cofactor) and the C-terminal domain is an RNA helicase. NS5 contains a methyltransferase (MTase) at the N terminus and an RNA-dependent RNA polymerase (RdRp) at the C terminus.[148]
RNA elements	NS5 polymerase domain helps to synthesize a transient double-stranded replicative RNA intermediate which is composed of viral plus- and minus-strand RNAs. The newly synthesized minus strand serves in turn as a template, allowing the RNA-dependent RNA polymerase to synthesize additional plus-strand genomic RNA.[182–186]
Nucleus	Either not expressed or relevance unclear fetal/infantile disease
Nucleosome	Either not expressed or relevance unclear fetal/infantile disease
DNA	No DNA genome exists
RNA	The dengue virion contains a single-stranded, positive-sense RNA genome of approximately 11 kb which is translated into a large polyprotein during the infectious life cycle.[7,180]
Genome-associated polyprotein	RNA genome of dengue virus is translated into a large polyprotein which in turn is processed by cellular and viral proteases into three mature structural proteins (C, prM, and E) and seven nonstructural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5).[180]
DNA polymerase	Either not expressed or relevance unclear fetal/infantile disease
RNA polymerase	The C-terminal region of NS5 has five amino acid sequence motifs which form the signature of RNA-dependent RNA polymerases (RdRps). Viral replication begins with the synthesis of minus-strand RNA from the positive-strand RNA genome, which then acts as a template for the formation of plus-strand RNA genomes. Production of new viral particles is catalyzed by the NS5 RNA-dependent RNA polymerase.[182]
Reverse transcriptase	Either not expressed or relevance unclear fetal/infantile disease
Head	Either not expressed or relevance unclear fetal/infantile disease
Base plate	Either not expressed or relevance unclear fetal/infantile disease
Integrase	Either not expressed or relevance unclear fetal/infantile disease
Tail	Either not expressed or relevance unclear fetal/infantile disease
Tail fiber	Either not expressed or relevance unclear fetal/infantile disease
Neck	Either not expressed or relevance unclear fetal/infantile disease

Wolbachia, an obligate intracellular bacterium, which lowers the susceptibility to infection by DENVs.[171–174]

Protective Measures against Mosquito Bites: This include careful use of insect repellents, wearing long-sleeved shirts and long pants, and control of mosquitoes inside and outside the home. Repellents containing DEET (name derived from DET in *N,N*-diethyl-meta-toluamide) are generally considered safe if used in only necessary amounts. These should not be applied on the face and around the eyes.

Detailed information for some of the viral components is listed in Table 1.

Future Directions

Future efforts should be directed toward the development of antiviral agents for the management of dengue. In addition, there should be an emphasis on planned urbanization with the escalation of efforts toward mosquito control and vaccine development.

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CASE SERIES

Spinal Ultrasound: A Safe and Valuable, but Underutilized Imaging Modality to Evaluate Epidural Hematomas in Infants

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Abstract

This paper aims to highlight the utility of spinal ultrasound as a valuable and safe diagnostic tool for spinal epidural hematomas in neonates and young infants. The accessibility, cost-effectiveness, and accuracy of spinal ultrasound make it an appealing alternative to magnetic resonance imaging (MRI). However, despite its potential benefits, spinal ultrasound remains underutilized in clinical practice. In this paper, we present a case study where spinal ultrasound successfully diagnosed a spinal epidural hematoma in a neonate. Additionally, a comprehensive review of current literature demonstrates a consensus on the advantages of spinal ultrasound for assessing spinal lesions in young infants and neonates. The findings of this study emphasize the importance of incorporating spinal ultrasound into clinical practice for more timely and convenient diagnosis of suspected spinal epidural hematoma in neonates and young infants.

Keywords: Accessibility, Accuracy, Anticoagulation, Antiplatelet therapy, Blood dyscrasias, Cauda equina nerve roots, Coagulopathies, Conus medullaris, Cost-effectiveness, Cost-effective, Epidural anesthesia, Evaluation, Hyperechogenic epidural fat, Hypoechogenic filar cyst, Infant, Imaging, Lumbar puncture, Newborn, Neonate, Neoplasms, Pediatrics, Pregnancy, Spinal hematoma, Spinal epidural hematomas, Epidural venous plexus, Spinal surgery, Spinal ultrasound, Thrombocytopenia, Time-efficient, Trauma, Tubular epidural fluid, Vascular malformations.

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Key Points

- Spinal ultrasound is increasingly recognized for its utility as a valuable and safe diagnostic tool for spinal epidural hematomas in neonates and young infants.
- The accessibility, cost-effectiveness, and accuracy of spinal ultrasound make it an appealing alternative to magnetic resonance imaging (MRI).
- Despite all its potential benefits, spinal ultrasound remains underutilized in clinical practice. In this paper, we present a case series where spinal ultrasound was successfully used to diagnose spinal epidural hematomas in young infants.
- In this article, we present a summary of the currently available literature, which shows an emerging consensus on the advantages of spinal ultrasound for assessing spinal lesions in these patients.

Introduction

Spinal epidural hematoma (SEH) is a serious condition characterized by the accumulation of blood in the epidural space surrounding the spinal cord. Although the incidence of this condition is low, symptomatic SEH is considered a surgical emergency due to the risk of minor or major permanent neurological deficits secondary to mass effect on the adjacent neural structures. Some asymptomatic cases of SEH can be managed conservatively, but these infants also need careful monitoring and follow-up.[1] Spinal epidural hematoma can also occur as a complication of spinal surgery or procedures, such as lumbar puncture and epidural anesthesia. Other risk factors include trauma, anticoagulation, neoplasms, coagulopathies, and vascular malformations. Magnetic resonance imaging is currently considered the first-line imaging modality for diagnosing SEH, but it is expensive and can be time-consuming, particularly in infants because of the need for sedation[2,3] Spinal ultrasound, on the contrary, can be performed quickly at the bedside, is more cost-effective, and has been shown to accurately diagnose acute spinal pathologies in neonates and young infants.[4,5]

In this paper, we present a case series of neonatal spinal epidural hematomas diagnosed by spinal ultrasound. Additionally, we have

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reviewed the existing literature on the utility of spinal ultrasound for the diagnosis of SEH in neonates and young infants with additional examples. The primary objective of this paper is to raise awareness among neonatologists, pediatricians, and radiologists regarding the advantages of spinal ultrasound as a safe and readily available imaging modality in this specific patient population. By highlighting its potential benefits and summarizing the relevant research, this paper aims to promote the appropriate use of spinal ultrasound for the timely and accurate diagnosis of this rare pathology.

Case Series

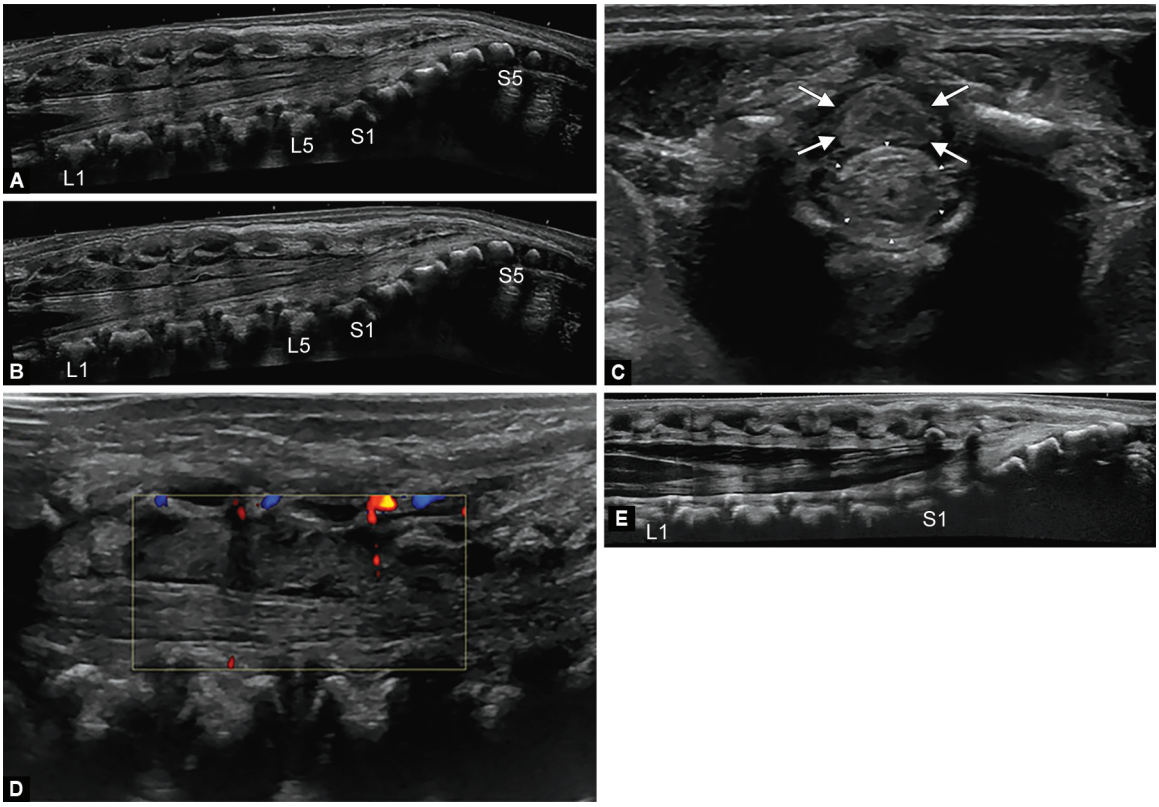
Fig. 1: A 2-week-old female was imaged after lumbar puncture to rule out meningitis. Panels A and B show sagittal, and panel C shows axial imaging of the lumbosacral spine. A mildly hyperechogenic, mildly lobulated epidural hematoma was seen dorsal to the dural sac, which appeared compressed by the hematoma (outlined in panel B). The hypoechogenic distal tip of the conus medullaris as well as the mildly hyperechogenic cauda equina nerve roots were separated from the epidural hematoma by a small sliver of hypoechogenic cerebrospinal fluid. Sagittal color-coded Doppler sonography (C) confirmed that the epidural hematoma was not perfused. Axial ultrasound (D) confirmed the epidural location of the hematoma (large arrows), dorsal to the displaced and mildly compressed dural sac (arrowheads). Sagittal ultrasound (E) of a normal distal spinal canal showed that the hypoechogenic conus medullaris and the mildly hyperechogenic cauda equine nerve roots were surrounded by a wide layer of hypoechogenic cerebrospinal fluid. The epidural space was overall narrow and was filled with a small amount of hyperechogenic epidural fat

Fig. 2: A 6-week-old boy was imaged after lumbar puncture. Sagittal ultrasound (A) of the distal lumbo-sacral spinal cord revealed a homogeneous hyperechogenic tubular epidural hematoma (arrows) displacing the dural sac anteriorly. The tip of the conus medullaris was intact (small arrow). Two axial ultrasound images (B and C) show the dura separating the epidural hematoma from the dural sac (arrows). The spinal cord/conus medullaris (*) and cauda equina nerve roots (+) were seen in the center of the anteriorly displaced dural sac

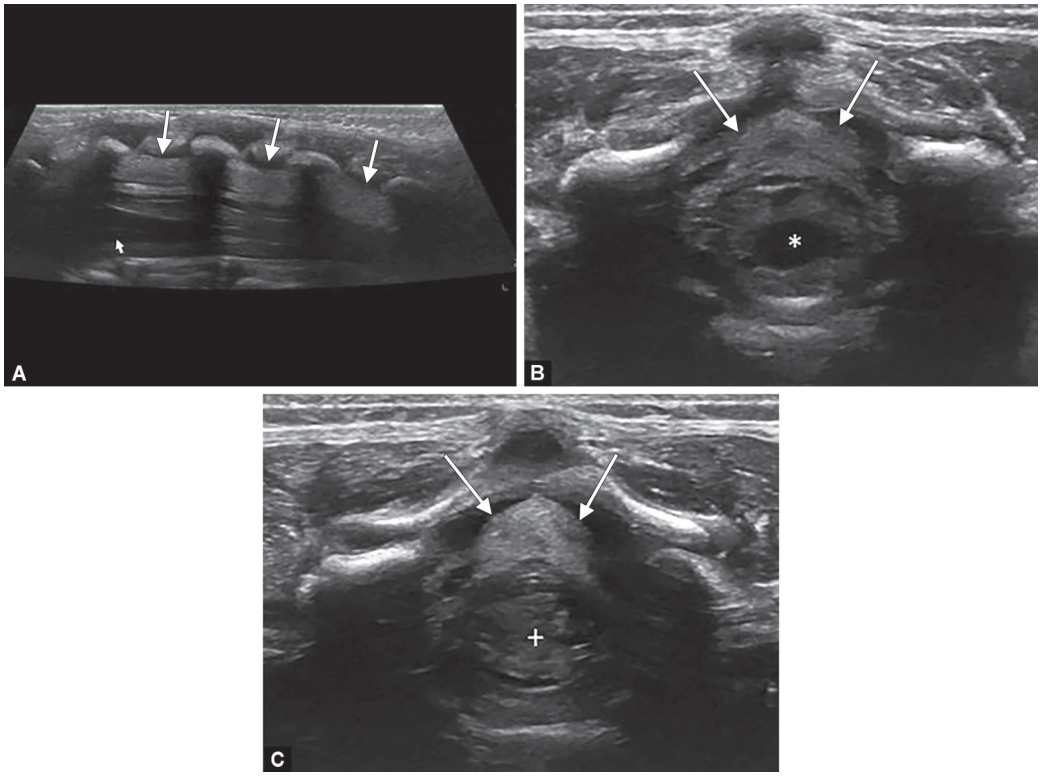
Fig. 3: Sonographic imaging of a 4-week-old boy after lumbar puncture. Sagittal ultrasound of the distal lumbo-sacral spinal cord revealed an ovoid heterogeneous hyperechogenic low sacral epidural hematoma (arrow). There was no appreciable mass effect on the distal dural sac

Fig. 4: A 4-day-old girl was imaged after lumbar puncture. Sagittal ultrasound and sagittal color-coded Doppler ultrasound images of the distal lumbo-sacral spinal cord revealed (A) a mixed hyper- and hypoechogenic ovoid epidural hematoma (arrows), which resulted in mild anterior displacement of the dural sac. The heterogeneity was most likely secondary to blood sedimentation, leveling within the epidural hematoma. The axial color-coded Doppler ultrasound image (B) did not show any perfused vessels within the hematoma

Fig. 5: Ultrasound imaging of a 6-day-old boy imaged after lumbar puncture. Sagittal and axial anatomical (A), and matching color-coded Doppler ultrasound images (B) of the lumbo-sacral spinal cord revealed a tubular hyperechogenic epidural hematoma (arrows) extending along the distal lumbar spinal cord, conus medullaris and cauda equina. Follow-up matching images (C and D) 3 days later showed progressive demarcation of the hematoma with increased



Figs 1A to E



Figs 2A to C

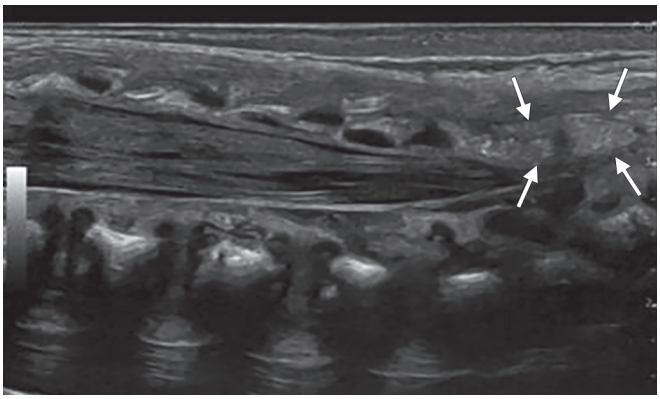
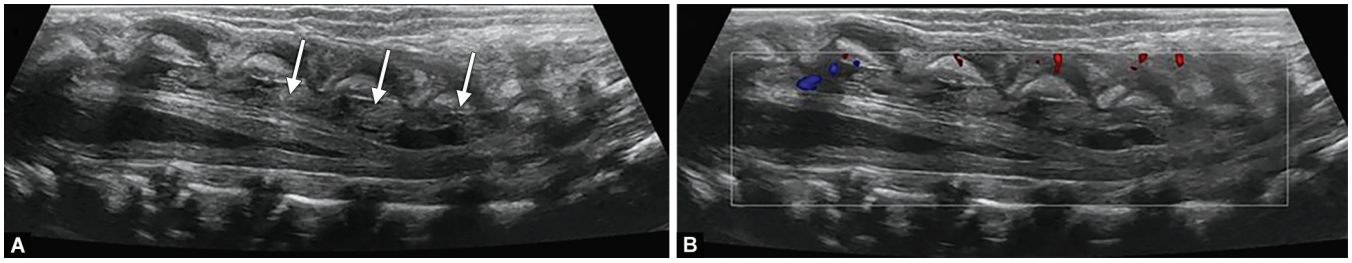
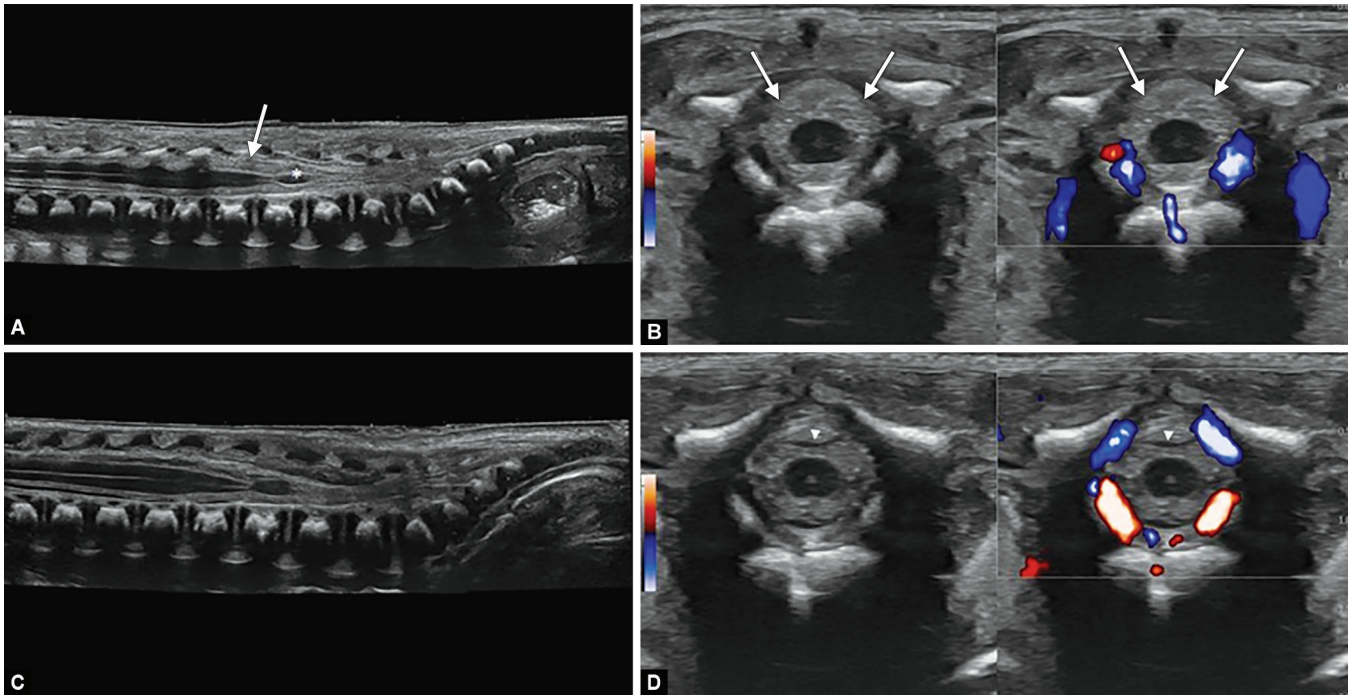


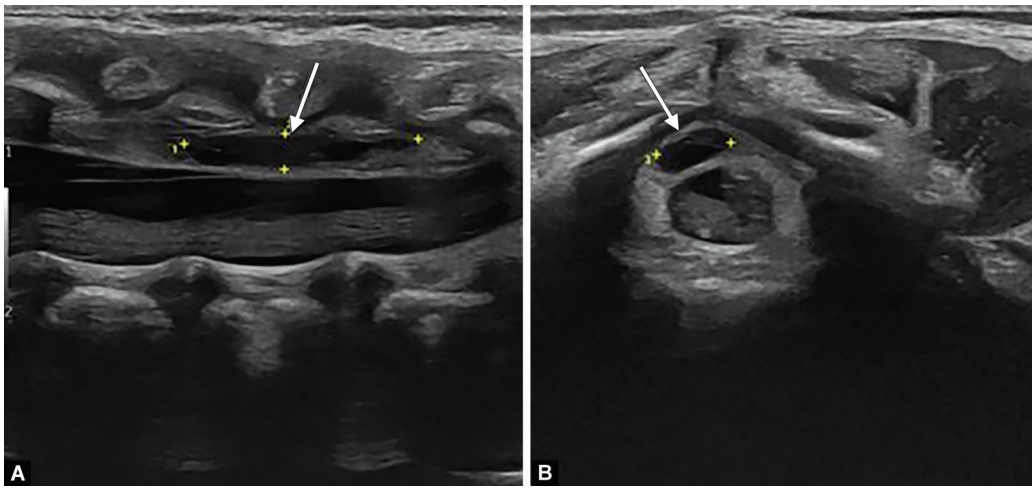
Fig. 3



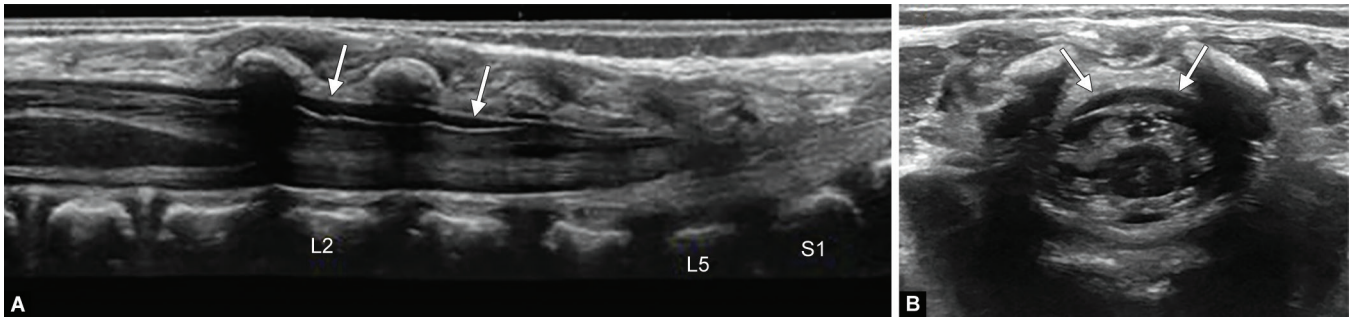
Figs 4A and B



Figs 5A to D



Figs 6A and B



Figs 7A and B

depiction of the hypoechogenic dura (arrowheads). A hypoechogenic filar cyst (*) was noted incidentally in its typical location

Fig. 6: A 4-week-old boy was imaged after lumbar puncture. Sagittal (A) and axial (B) ultrasound images of the distal lumbo-sacral spinal cord revealed a predominantly hypoechogenic ovoid epidural hematoma (arrows) slightly off-midline, along the right hemi-circumference of the dural sac. The hypoechogenicity indicated the chronic nature of the hematoma with progressing liquefaction and resorption

Fig. 7: A 3-week-old girl after lumbar puncture. Sagittal ultrasound (B) of the distal lumbo-sacral spinal cord revealed a homogeneous hypoechogenic tubular epidural fluid collection (arrows) causing mild anterior displacement of the dural sac. The hypoechogenic fluid collection represented cerebrospinal fluid leakage along the dural puncture site from the dural sac into the epidural space. The axial ultrasound image (B) confirmed the epidural CSF collection (arrows)

Discussion

Spinal epidural hematoma is a known complication of invasive spinal procedures such as lumbar puncture. Most of these cases are asymptomatic and resolve spontaneously. The risk of spinal hemorrhage after invasive procedures increases with anatomical factors, such as vertebral abnormalities, multiple attempts at needle placement, and the use of anticoagulation or antiplatelet therapy. Other risk factors include trauma, pregnancy, coagulopathies, blood dyscrasias, and thrombocytopenia. The source of bleeding in SEH remains a topic of debate. Most authors endorse that the bleeding likely originated from the epidural venous plexus, a low-pressure network of large, thin-walled veins located in the epidural space that may be easily ruptured. Others suggest that the free arteries running near the venous plexus may be responsible for the bleeding.[1,6]

Most cases of SEH resolve spontaneously without causing significant symptoms, as was the case with our patients. Symptomatic SEH is infrequent and accounts for <1% of space-occupying spinal lesions.[7] Asymptomatic SEH can typically be managed conservatively with close monitoring involving serial neurological exams and imaging to monitor for changes in size. Symptomatic SEH, on the contrary, can cause life-threatening spinal cord compression and requires urgent surgical decompression. Common symptoms of symptomatic SEH include back pain, paresthesias, paresis, and urinary incontinence.[1,8,9] However, infants are more likely to present with less specific clinical features. Neurological deficits can be more difficult to recognize in these patients, potentially leading to delays in diagnosis.[8] Thus, prompt and accurate imaging is crucial for early diagnosis and appropriate management.

Spinal ultrasound can efficiently and accurately diagnose spinal hematomas. Our case series shows that ultrasound can be a useful initial diagnostic modality, which may also be used for monitoring during follow-up. Compared with magnetic resonance imaging, the current first-line modality, spinal ultrasound offers more widespread accessibility, portability, and the ability to be performed quickly at the bedside.[4,10] Additionally, there have been remarkable advancements in ultrasonography in recent years due to the introduction of new-generation high-frequency ultrasound scanners. This new technology has significantly enhanced the image quality of ultrasound, effectively placing its diagnostic value at par with that of MRI in certain situations.[11–13] Furthermore, another important advantage of ultrasound over MRI, especially in young infants, is that ultrasound examinations can be performed without the need for sedation. Unlike MRI, which often requires children to remain still for an extended period inside a confined space, ultrasound is a noninvasive and generally well-tolerated procedure that can be conducted while the child is awake. This eliminates the need for sedation or anesthesia, reducing potential risks and complications. This not only improves patient comfort but also provides a safer and more convenient option.

Despite its advantages, there has been a scarcity of literature detailing the use of ultrasound for the diagnosis of SEH. Leadman et al.[14] described a successful case of SEH diagnosed using ultrasound in 1988. Since then, a few other cases have been reported.[5] Nevertheless, the existing literature suggests that spinal ultrasound may be an optimal initial diagnostic modality for the assessment of SEH in infant-aged patients. In the first 6 months of life, the human spine is primarily cartilaginous, creating an acoustic window that is ideal for transmitting ultrasound waves. The quality of ultrasound images decreases after this time-period as the progressive ossification of the spine obscures the acoustic window. Therefore, neonates and young infants are an ideal patient demographic for the use of ultrasound for the assessment of spinal lesions; it can be very useful for the evaluation of spinal lesions, such as spinal dysraphisms, tumors, trauma, and vascular malformations.

Spinal ultrasound findings in neonates and young infants show a strong correlation with MRI. Rohrschneider et al. compared spinal ultrasound and MRI findings in 24 pediatric patients (mean age 5.5 months); ultrasound correctly diagnosed spinal abnormalities in all patients.[15] Similar findings were reported in smaller studies.[16,17] However, it is important to know the limitations of spinal ultrasound compared with MRI. MRI offers highly detailed anatomical images with excellent soft tissue contrast, making it particularly valuable for assessing complex spinal pathologies and providing a more comprehensive visualization of the anatomy. In addition, ultrasound is more operator-dependent and may have limitations in visualizing deep-seated structures or differentiating certain soft tissue structures.[15] In more complex cases or situations where adequate visualization cannot be obtained using ultrasound, MRI may be more appropriate.

Conclusion

Spinal ultrasound is a painless, highly feasible, accessible, accurate, cost-effective, time-efficient, and risk-free modality for the diagnosis of spinal epidural hematomas and other lesions. It offers several advantages over MRI in the diagnosis of neonates and young infants and allows for prompt and convenient diagnosis. Neonatologists, pediatricians, and radiologists should be familiar with this easily-available imaging tool to evaluate the spine and spinal cord.

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