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GNS Bangladesh Newborn Foundation
GNS Foundation of Germany
Global Newborn Society Foundation of Italy
Mongolian Association of Obstetrics Gynecology and Neonatology
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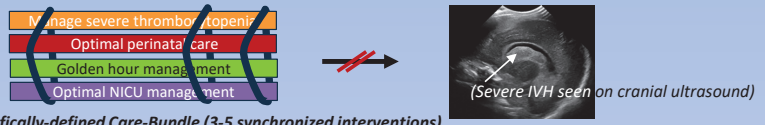
Neonatology Branch of the Chilean Pediatric Society
Carlo GNS Center for Saving Lives at Birth
Dudeja GNS Center for Infectious Diarrheal Diseases
Anatolian Midwives Association
The Organization, First Breaths of Life

Highlighted articles:

- **A Care-Bundle to Prevent Germinal Matrix-Intraventricular Hemorrhage (GM-IVH) in Neonates**

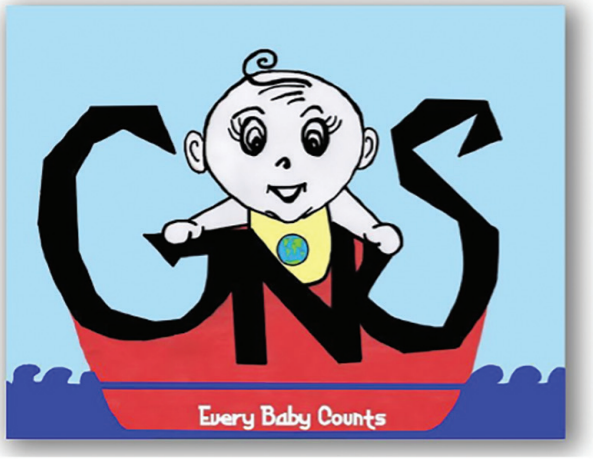


Specifically-defined Care-Bundle (3-5 synchronized interventions)

- **Down Syndrome is the Leading Indication for Late-Stage Termination of Pregnancy in Mongolia**
- **A Primer on Epigenetic Changes: The More We Know, the More We Find in Fetuses and Infants**

Also available online at
https://www.globalnewbornsociety.org/our-scientific-journal-newborn

Bibliographic Listing:
J-Gate, Scilit, WorldCat,
PORTICO, ICMJE



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 eyelashes, 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, worldwide 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 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 cast 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 online at https://www.globalnewbornsociety.org/intructions-for-authors. The manuscripts can be submitted via the online manuscript submission system.

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Contents

EDITORIAL

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Healthcare Bundles are Potentially Important in Neonatal Care as Many Disorders are Temporally Clustered

The late fetal and neonatal periods carry a high risk of morbidity and mortality.[1] Each year, we lose nearly 5 million 3rd trimester fetuses and neonates.[2] And as we have mentioned in our previous issues, babies do not talk,[3] or vote,[4] and so, need help.[2] We also need to remind ourselves that since we still do not clearly understand the exact etiology of most neonatal disorders, these need to be viewed as multi-manifestation 'syndromes', not 'diseases'. Hence, to develop treatment strategies that will remain effective despite the clinical variability, we need cohorts from large geographic/climatic regions and with multiple races/ethnicities. A truly multinational group of care-providers drawn from all over the world can also help understand regional differences in clinical approach. The Global South, with its higher fertility rates and limited access to healthcare facilities, definitely needs to be represented.[5–8] These peri-equatorial/tropical regions could also have a greater proportion of cases with underlying/confounding infections, which need to be studied.[9] All these issues need consideration in healthcare planning.[10] The need for access to updated data about healthcare, outcomes, and changing economic status cannot be over-emphasized.[11]

One possible solution for the multidimensional health problems of premature/critically ill infants could be in the application of healthcare "bundles", a concept introduced by the Institute of Health Care Improvement (IHI). These bundles refer to simultaneous application of 3–5 evidence-based or traditionally accepted interventions to prevent/treat specific clinical disorders in all eligible patients.[12–15] The concurrent use of multiple treatment modalities is attractive in premature/critically ill infants as they typically show the highest severity of illness during the early postnatal period and/or at specific corrected gestational/post-conceptional ages.[12,13,16–21] Initial studies have shown an encouraging evidence for this approach.[22] Therefore, the leadership of the Global Newborn Society (GNS) has requested clinician experts from all over the world to develop and then evaluate this approach in various neonatal disorders. A short acronym was chosen to describe this evolving database: LAYA - ***L***ooking ***A***t ***Y***our practices in ***A***pplication.[23]

Our journal, the newborn aims to cover fetal/neonatal problems that begin during pregnancy, at the time of birth, or during the first 1000 days after birth. The movement is growing; since our last issue 3 months back, this journal has now been adopted by 13 more organizations as their official mouthpiece. These include the Association of Pediatricians of Uzbekistan, GNS Cardiology Association of Iraq, Iranian Forum for Infant Nutrition, Nepalese Association for Newborn Health, GNS Forum for Transgenerational Inheritance, PreemieWorld Foundation, GNS Forum for Data Analytics, GNS Forum for Nanomaterials, Neonatology Branch of the Chilean Pediatric Society, Carlo GNS Center for Saving Lives at Birth, Dudeja GNS Center for Infectious Diarrheal Diseases, Anatolian Midwives Association, and the organization, First Breaths of Life. Thus, we now represent a total of 34 groups for their official communications. We will share scientific data, viewpoints, and clinical observations relevant to the care of all ill infants and also focus on important concerns related to Down syndrome, autism, infant nutrition, brain injury, and care of infants in remote areas.

As in our previous issues, we again present 8 important articles here (**Fig. 1**). We bring to you the second in our series of healthcare bundles—this one is focused on Germinal Matrix-Intraventricular Hemorrhages (GM-IVHs).[24–26] GM-IVHs are seen in nearly a 1/3rd of all very-low-birth-weight infants within the first 72 hours after birth, and hence represent an important opportunity for bundled precautionary measures.[24–31] Ben Ayad et al.[32] have carefully evaluated antenatal measures such as the administration of steroids and magnesium sulfate, perinatal measures such as delayed cord clamping and management of thrombocytopenia and/or coagulopathy,

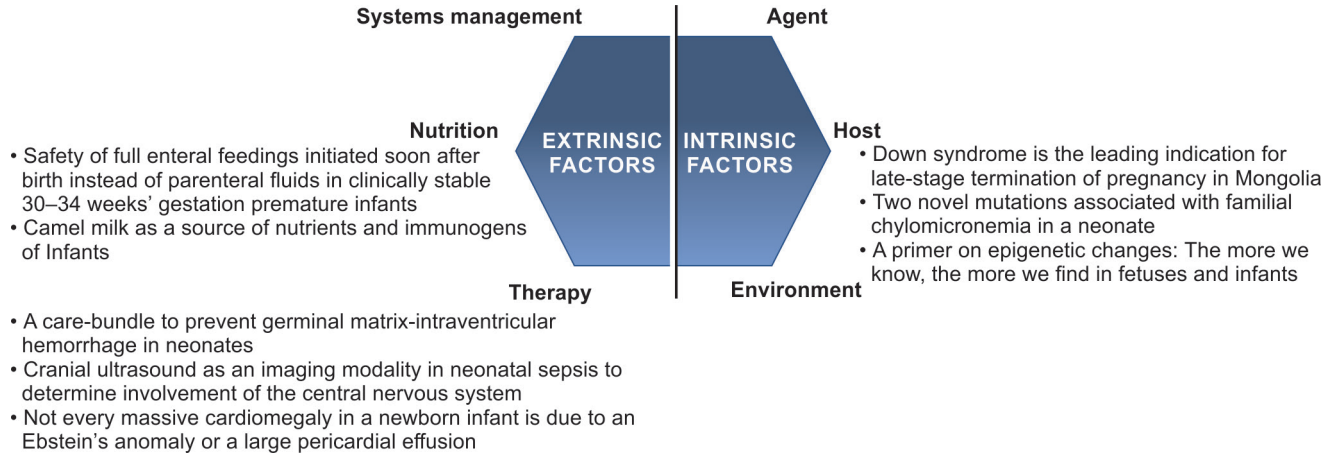

Fig. 1: Areas of focus in the *newborn*, Volume 3, Issue 3. We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health — those originating in therapy, nutrition, and systems management are shown. This issue covers 3 nodes, namely host factors, treatment/monitoring systems, and nutrition.

and postnatal maintenance of a midline head position, safe transport, efforts to avoid hemodynamic instability, cautious endotracheal suctioning, limit blood withdrawals, and avoid routine flushing of intravascular lines.[32–38] They have proposed a 4-point bundle to prevent GM-IVH in premature infants: (a) appropriate neonatal resuscitation with, if possible, delayed cord clamping; (b) Golden-hour care; (c) gentle care of outborn infants including safe transport and avoiding hemodynamic instability; and (d) cautious management of perinatal thrombocytopenia.[39–45] We will report compliance and the impact on the incidence/severity of GM-IVH in the next 3–5 years.

Badarch et al.[46] examined the medical records of 45,095 women in Ulaanbaatar, Mongolia, to identify the most frequent indications for termination of pregnancy for fetal anomalies (TOPFAs).[47–49] Timely detection of these disorders may help make informed decisions to choose either safe terminations or well-timed fetal procedures for rehabilitation.[50] This information may also be important for appropriate genetic testing to assess the risk of recurrence in later pregnancies. They identified 156 TOPFAs (34.5 per 10,000 pregnancies) in this cohort. These infants with fetal/congenital anomalies were compared with 312 healthy controls to evaluate maternal risk factors. Down syndrome was the most frequent reason (25%) for termination of pregnancy, followed by detection of multiple congenital anomalies (16%), cleft lip/palate (10.9%), and anomalies of the central nervous (9.6%) or musculoskeletal system (9.6%). Maternal age >35 years, higher education, closely spaced successive pregnancies, and previous history of abortion (s) were linked with a higher likelihood of birth defects.

Singh and coworkers[51] have provided an interesting review of epigenetic changes, an emerging area of study focused on heritable information without alterations in the DNA sequence. [1,8,27] The most frequently seen epigenetic changes include DNA methylation, changes in gene expression due to noncoding ribonucleic acids (RNAs), and post-translational modifications of histone proteins.[56] During this period, atypical metabolic reprogramming induced by extrinsic factors such as allergens, viruses, pollutants, diet, or microbiome might also alter cellular metabolism and immune responses.[9] Understanding the variability in epigenetic marks could help understand the pathogenesis of many neonatal disorders, develop new therapeutic approaches, and even explain some of the pleiotropy in syndromic disorders.

Garegrat and her team[52] have reviewed the importance of point-of-care cranial ultrasound (POCUS) in detection of central nervous system (CNS) involvement in neonatal sepsis. POCUS findings can show signs of meningitis, brain abscesses, changes in the spinal cord, and alterations in cerebral blood flow.[53–56] Color doppler may give clues regarding the location of the extra-axial fluid collection(s).[57,58] It can even provide some clues for early identification of fungal and viral infections.[59–66] All this information can aid in appropriate management. The ease of usage, safety, and a short turn-around time makes ultrasound superior to the other imaging techniques in neonatal infections.[60]

Mohammadabadi and Kumar[67] have reviewed the nutritional value of camel milk, which is widely used for feeding infants in arid and semiarid regions. Even though camel milk represents only 0.36% of global milk production, it has several notable characteristics in its composition. It contains 3.4% protein, 4.4% lactose, 3.5% fat, high levels of vitamin C, a favorable ratio of unsaturated to saturated FAs.[68] There are more long-chain FAs, linoleic acid, and unsaturated fatty acids. Camel milk also contains important immune factors such as the VHH (single variable domain on a heavy chain) antibodies/nanobodies, which are much smaller than conventional antibodies and can stimulate immune responses.[69] Camel milk is also hypoallergenic as it does not contain β-lactoglobulin.[70] Finally, its probiotic bacteria and bioactive peptides can reduce cholesterol absorption,[71] further enhancing its health benefits.

Hoyos and Vasquez-Hoyos[72] have reported a quality-improvement effort where they tested the safety and efficacy of enteric feedings beginning within 2 hours after birth in infants born at 30–34 weeks' gestation. They administered oral/nasogastric milk feedings at 70–80 mL/kg/day divided every 3 hours, with 5 mL increments every 12–24 hours until 200 mL/kg/day was achieved. This effort differs from current practice in most centers where such infants are maintained on parenteral fluids for variable periods until hemodynamic stability is confirmed. In this QI effort, early enteral feedings were well tolerated with stable growth and biochemical parameters. The investigators inferred that routine use of parenteral fluids is not necessary in the initial management of these infants.

Jha et al.[73] have described a 12-day-old infant who was presented with respiratory distress, hepatosplenomegaly, and *lipemia retinalis*.[74] The laboratory noted that his sera were unusually viscous and turned opaque milky-white within minutes. There was chylomicronemia with high triglyceride and cholesterol levels. Genetic analysis showed a novel homozygous mutation in the lipoprotein lipase (LPL) gene and a heterozygous missense variation in the sterol regulatory element-binding transcription factor 2 (SREBF2).[75,76] There was rapid improvement with dietary modifications. This case reminds us yet again of a need to be cognizant of non-infectious causes of neonatal respiratory distress. Timely diagnosis and intervention can improve outcomes.

Finally, Barrios et al.[77] have described a term infant with respiratory distress and a massive cardiomegaly noted on a chest radiograph. They considered the usual differential diagnosis of tricuspid valve malformations as in Ebstein's anomaly, large pericardial effusions, fetal cardiomyopathy, and cerebral/hepatic arterio-venous malformations.[78–87] However, magnetic resonance imaging and computed tomography showed a large cyst in the left hemithorax. The heart and major vessels were all normal in size. A left posterolateral thoracotomy was performed to remove the cyst; histopathology showed features of bronchopulmonary foregut malformations.[88,89] We need to consider a wider list of entities in the differential diagnosis of a massively enlarged cardiac silhouette in an infant with respiratory distress.

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ORIGINAL RESEARCH

A Care-bundle to Prevent Germinal Matrix–Intraventricular Hemorrhage in Neonates

The *LAYA** Group of the Global Newborn Society
**L*ooking *A*t *Y*our practices in *A*pplication

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Abstract

Germinal matrix-intraventricular hemorrhages (GM-IVHs) can be seen in up to 25–30% of premature infants. These are associated with a major psychological, social, and financial challenge for care-providers and families caring for premature infants all over the world. The severity is usually classified based on the location and volume vis-à-vis that of the cerebral ventricles, including (A) Grade I GM-IVHs localized in the germinal matrix; (B and C) Grade II and III hemorrhages occupying less than and more than 50% of the ventricular cavities, respectively; and (D) Grade IV IVHs that extend into the surrounding parenchyma with/without a periventricular hemorrhagic infarction (PVH). Germinal matrix-intraventricular hemorrhages have been associated with impaired neurodevelopment (17.5%), static physical disabilities in cerebral palsy (7–63%), deafness (8.6%), and blindness (2.2%). Considering the complex etiopathogenesis of GM-IVH and the fact that most of these events occur within a temporally-delimited period of the first 72 hours after birth, there is increasing interest in the structured application of 3–5 well-accepted preventive measures as a quality improvement (QI) "care bundle" during the high-risk period. In this article, we have described the evidence on which our GM-IVH bundle is based. We have carefully evaluated antenatal factors such as the history of having received steroids and magnesium sulfate, perinatal measures such as delayed cord clamping, management of thrombocytopenia and/or coagulopathy, and postnatal measures such as maintaining a midline head position, cautious endotracheal suctioning and blood withdrawals, and avoidance of routine flushing of intravenous and arterial lines. Based on the strongest evidence and practice consensus, we have adopted a 4-point bundle to prevent GM-IVH in premature infants: (A) Appropriate neonatal resuscitation with, if possible, delayed cord clamping; (B) Golden-hour care; (C) Gentle care of outborn infants including safe transport and avoiding hemodynamic instability; and (D) if needed, management of perinatal thrombocytopenia and coagulopathy. In the next 3–5 years, we will report compliance and changes in the incidence/severity of GM-IVH at our centers.

Keywords: Antenatal corticosteroids, Delayed cord clamping, Germinal matrix-intraventricular hemorrhage care-bundle, Golden hour, Implementation science, Institute of health care improvement, Neonate, Newborn, Periventricular hemorrhage, Tocolytics.

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Keypoints

Germinal matrix-intraventricular hemorrhage (GM-IVH) is a well-known co-morbidity and mortality of prematurity, especially in very-low-birth-weight (VLBW) infants.

1. A care bundle is a structured, organized attempt to develop protocols to prevent/alleviate possible major risk factors for a disorder.
2. Germinal matrix-intraventricular hemorrhage is a complex, multifactorial disorder that typically occurs within a temporally-delimited period of the first 72-hours after birth, and therefore, developing a clinical care bundle to mitigate known risk factors is logical to improve neonatal outcomes.
3. In this article, we have proposed a 4-point bundle to prevent GM-IVH in VLBW infants: (A) Appropriate neonatal resuscitation, and if possible, delayed cord clamping; (B) Golden-hour care for inborn infants; (C) Gentle care of out born infants, including safe transport and avoiding hemodynamic instability; and (D) if needed, management of perinatal thrombocytopenia and coagulopathy.

Introduction

Germinal matrix-intraventricular hemorrhage (GM-IVH) is an important cause of morbidity and mortality in extremely premature/very low-birth-weight (VLBW) infants.[1–3] The most important predisposing factors in these patients include the fragility of the premature cerebral vasculature and fluctuations in cerebral blood flow.[3,4] Despite improvements in the overall survival rates, the incidence of moderate-to-severe GM-IVH has remained the same among preterm infants in the last few decades. This might be partially explained by improved neonatal care, with increasing survival of ELBW infants born at 22–24 weeks' gestation.[1,5] Indeed, low gestational age is one of the most important risk factors for developing GM-IVH.[6–8] There are some geographic variations in

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the incidence of GM-IVH; globally, population-based studies show the incidence of Grade I–II IVH as ranging between 5 and 19% and Grade III–IV at 5–52% (Europe: 5–52%; North America: 8–22%; Asia: 5–36%; Oceania: 8–13%).[6]

In this paper, we have described how we developed our therapeutic "bundle" to lower the risk of GM-IVH in premature infants.[9–11] As defined by the Institute for Healthcare Improvement (IHI), a therapeutic bundle is the concomitant application of 3–5 evidence-based interventions to improve outcomes in a defined subset of patients.[12–14] Although the development of bundles is always an evolving process with scope for further improvement, it is a major advance in our understanding of the impact of therapeutic interventions. There is a need for a better understanding of the scientific principles, continued compliance, and regular measurements to define/refine these interventions continuously.[9,14–16] This section outlines our efforts to define, evaluate, and refine this care bundle to prevent GM-IVH.

Defining GM-IVH

Germinal matrix-intraventricular hemorrhage signifies bleeding in the brain, primarily in the germinal matrix (GM) and then in the ventricles.[1,17,18] Abraham Towbin first identified it in 1968.[19] Grading systems were developed by Papile et al. in 1978 based on head CT scans, and later, several more classifications were developed; Papile and Volpe's classification is currently the most widely accepted all over the world.[20–22] A IV-grade classification system of GM-IVH based on the location and severity of hemorrhage is used most frequently: (A) Grade I hemorrhages are limited to the GM; (B) Grade II implies extension to lateral ventricles without ventricular dilatation; (C) Grade III indicates ventricular hemorrhage with ventriculomegaly; (D) Grade IV is ventricular hemorrhage with extension into the nearby brain parenchyma.[21] Arbitrarily, grades I-II are considered mild, whereas grades III-IV are considered severe GM-IVH.[23] Classifications based on parasagittal views on cranial sonography include 3 grades of severity: Grade I refers to GMH with no/minimal IVH (<10% of ventricular area); Grade II hemorrhage occupies 10–50% of the lateral ventricles; Grade III occupies >50% of these ventricles, usually with ventricular distention and periventricular echodense areas.[24]

The GM is a highly vascular area with abundant neuronal and glial precursor cells. Unlike other brain regions, the GM shows a high degree of angiogenesis, with immature vessels creating immature vessels. These immature vessels lack pericytes, exhibit immature basal lamina low in fibronectin, with less supporting matrix, and have astrocyte end-feet coverage deficient in glial fibrillary acidic protein (GFAP).[25,26] Consequently, the vasculature in the GM remains very fragile and vulnerable to rupture, with a high risk of hemorrhages. Premature infants also have limited cerebral autoregulation and often show fluctuations in cerebral blood flow due to respiratory or hemodynamic instability. Germinal matrix-intraventricular hemorrhage is associated with high mortality and adverse outcomes such as post-hemorrhagic hydrocephalus, cerebral palsy, epileptiform seizures, severe cognitive delays, and visual and hearing impairments. Many of these affected neonates may initially be asymptomatic and are diagnosed only on routine cranial sonograms. Many studies have evaluated antenatal measures like antenatal steroids, magnesium sulfate, and outreach programs perinatally like golden hour, delayed cord clamping, minimal handling, midline position, a nursing bundle of care, transportation care including in utero-transfer, neuroprotective bundle during transfer like avoiding noise, minimizing vibrations, keeping the head in the midline, and respiratory and hemodynamic management as singular measures. However, recent changes in practice have emphasized the potential value of a care bundle approach.

Defining Care Bundles

Care bundles are powerful tools used in modern medicine. These are formulated by applying evidence-based QI interventions that have been considered/proven beneficial in a specific disease or care process.[9] The IHI introduced the concept of "care bundles" mainly to improve medical care and adherence to evidence-based guidelines in intensive care units (ICUs). In this schema, 3–5 evidence-based or globally-accepted interventions are grouped to address specific aspects of patient care.[14] Unless contraindicated, care bundles are not limited to certain patient populations and are usually focused on correcting specific healthcare issues in one or more facilities. The goals are to improve consistency in treatment measures, adherence to best practices, improve patient safety, reduce complications, and lower healthcare costs, morbidity, and mortality. Healthcare bundles can be tailored for preventive medicine, improved clinical management, and/or to manage acute/chronic complications or sequelae. Even though all QI efforts have not been universally proven clinically useful and effective, the bundled application might show different results as the lack of application of one intervention might reduce the efficacy of another. This article reviewed current information supporting various therapeutic interventions and defined a GM-IVH care bundle.

GM-IVH Care Bundle

We have carefully evaluated antenatal factors such as the history of having received steroids and magnesium sulfate, perinatal measures such as delayed cord clamping, management of thrombocytopenia and/or coagulopathy, and postnatal measures such as maintaining a midline head position, cautious endotracheal suctioning and blood withdrawals, and avoidance of routine flushing of intravenous and arterial lines. Based on the strongest evidence and practice consensus, we have adopted a 4-point bundle to prevent GM-IVH in premature infants (Fig. 1): (A) Appropriate neonatal resuscitation with, if possible, delayed cord clamping; (B) Golden-hour care; (C) Gentle care of inborn and outborn infants including safe transport and avoiding hemodynamic instability; and (D) protocolization of respiratory management. In the next 3–5 years, we will report compliance and changes in the incidence/severity of GM-IVH at our centers.

Here is a detailed review of the most discussed preventive/therapeutic interventions:

- Coordinate perinatal care with obstetricians and outborn measurements:
  - Preterm premature rupture of membranes (PPROM) and chorioamnionitis:

Preterm premature rupture of membranes occurs in 2–3% of all pregnancies and is responsible for >25% of preterm births (PTB).[14,27] The definition of PROM in terms of duration is still debatable, but most centers accept 18 hours of ruptured membrane as a significant risk factor for early-onset infections. Existing information shows PPROM or PTB in earlier pregnancies, use of tobacco, presence of sexually transmitted diseases, and low body mass index (BMI) as risk factors for PPROM.[28,29] The underlying etiology of PROM is often unclear; multiple factors such as maternal age, parity, infections,

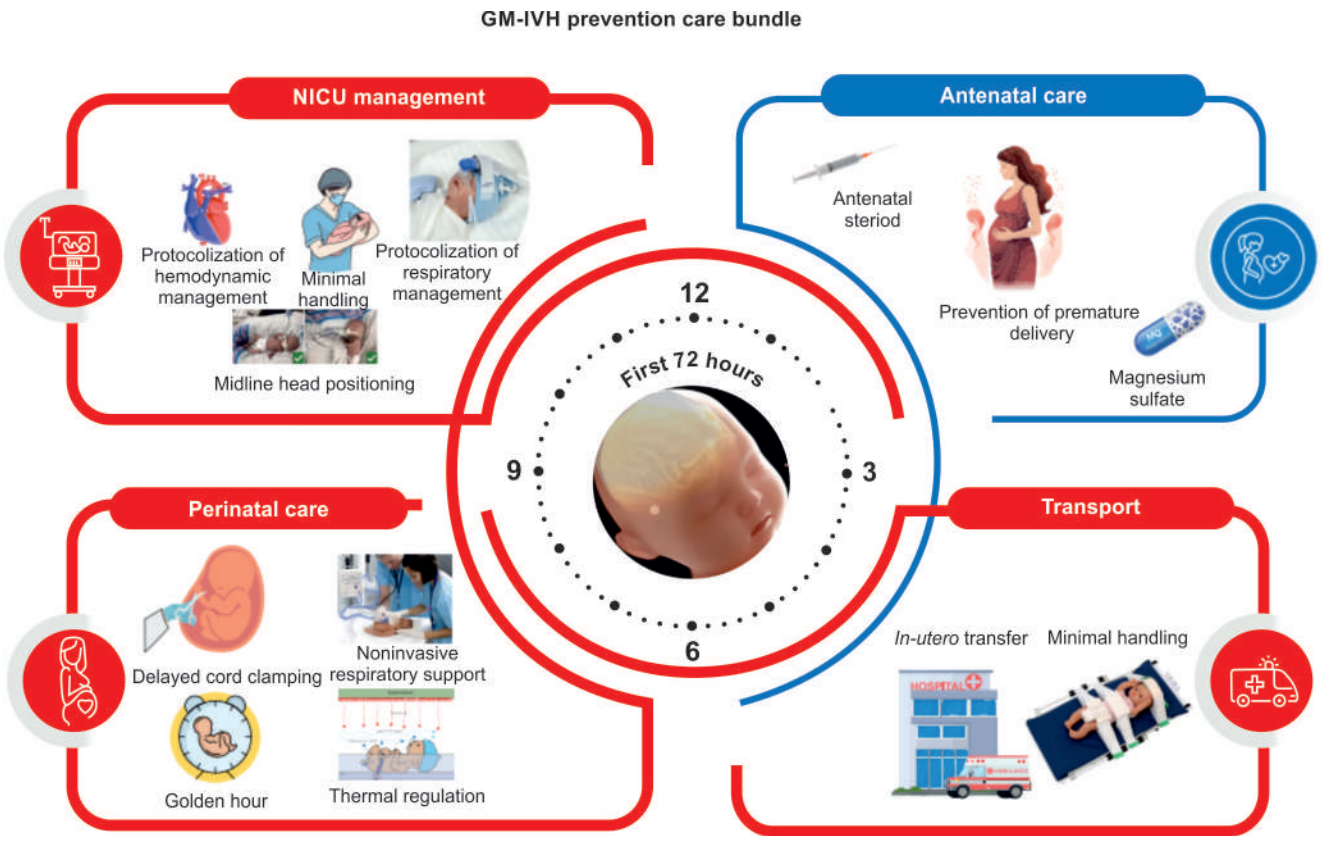


Fig. 1: Measures that are usually considered in developing protocols to prevent GM-IVH. We have selected 4 of these interventions to develop a care bundle: (A) Appropriate neonatal resuscitation with, if possible, delayed cord clamping; (B) Golden-hour care; (C) Gentle care of outborn infants, including safe transport and avoiding hemodynamic instability. The relationship with hemodynamic instability still requires more evidence; (D) Management of perinatal thrombocytopenia (not shown). These steps have been highlighted with red outliners. Several other measures may be helpful, but further evidence is needed

altered structural/functional integrity of the membranes, and genetic factors might be involved.[30,31]

The management of PPROM is usually focused on delaying delivery when possible and optimizing neonatal outcomes. Antibiotics can prolong the duration of pregnancy by preventing chorioamnionitis and consequent/concurrent neonatal infections.[32,33] Broad-spectrum antibiotics are recommended with PPROM as the infection is often polymicrobial and has several microbial agents of chorioamnionitis.[29,34]

Chang et al.[35] compared neonatal morbidity and neurologic outcome at 2 years after birth in a retrospective study of mothers with PPROM who delivered before 32 weeks' gestation and were treated with antibiotics. One hundred sixty-six women were included: 80 were treated with erythromycin and 86 with clarithromycin. The clarithromycin group had a higher median gestational age ($p = 0.005$), but fewer mothers had histopathologically-confirmed chorioamnionitis ($p = 0.004$). After adjusting for the confounding factors, the multivariable analysis showed the incidence of severe IVH (≥ Grade III) was lower in the clarithromycin group (GM-IVH; OR 0.23, 95% CI: 0.06–0.91; also, less BPD; OR 0.34, 95% CI: 0.13–0.90). They suggested that a clarithromycin-based regimen may be worth considering as a choice of erythromycin in PPROM patients.[35,36]

In a retrospective study, Lee et al.[37] compared perinatal outcomes in 314 patients with PPROM who were born at a gestational age <34 weeks and received either an anti-microbial regimen 1 (ampicillin and/or cephalosporins; $n = 195$, 1993–2003) or a regimen 2 (ceftriaxone, clarithromycin, and metronidazole; $n = 119$, 2003–2012). Intra-amniotic infection/inflammation was assessed by a positive amniotic fluid culture and/or an elevated amniotic fluid matrix metalloproteinase-8 (MMP-8) concentrations (>23 ng/mL). Germinal matrix-intraventricular hemorrhages and cerebral palsy (CP) were significantly lower in patients allocated to regimen 2 than regimen 1 (GM-IVH: 2.1 vs 19%, $p < 0.001$ and CP: 0 vs 5.7%, $p < 0.05$).[37]

These benefits have not been seen consistently across all cohorts. In a retrospective study involving 287 pregnant women with PPROM between 23 and 33^{+6} weeks' gestation, DiGiulio et al.[34] concluded that extended 7-day azithromycin administration was associated with significantly increased latency (>3 days) compared to those treated with a 2-day routine azithromycin regimen. There were no effects on other maternal or neonatal outcomes. However, a systematic review and meta-analysis showed no difference in the risk of severe GM-IVH following antibiotic treatment for PPROM (RR: 0.73; 95% CI: 0.42–1.26; $I_2 = 0\%$, 4 RCTs, $n = 893$ participants; certainty of Evidence/CoE: low).[35]

Antenatal Corticosteroid Therapy

Antenatal steroids (ANS) such as dexamethasone or betamethasone can lower the risk of GM-IVH in preterm infants. These protective effects likely involve multiple mechanisms: accelerated maturation of the cerebral vasculature with improved vasomotor responses; decreased need for respiratory support/increasing surfactant

production and consequently improving lung compliance, stabilization of alveolar and capillary membranes that lowers permeability and the risk of inflammation, and promotes structural support through pericyte recruitment. Steroids also likely suppress inappropriate expression of vasoactive mediators such as angiopoietin and vascular endothelial growth factors and, consequently, prevent dysregulated angiogenesis and associated formation of unduly fragile and immature capillary networks in the GM and beyond.[38–40] In addition, steroids may also have indirect protective effects that involve suppression of systemic inflammatory responses, improvements in respiratory function with less need for mechanical ventilation, and the systemic acid-base balance, all of which can potentially alter cerebral blood flow.[41] McGoldrick et al.[39] reported a reduced risk of GM-IVH following ANS administration (RR: 0.58; 95% CI: 0.45–0.75; 8,475 infants; 12 RCTs, CoE: moderate; 1.4% fewer, 95% CI: 0.8–1.8% fewer). Korcek et al.[38] also reported a lower risk for any GM-IVH (OR: 0.58; 95% CI: 0.39–0.85; $p = 0.006$) and for high-grade GM-IVH (OR: 0.36; 95% CI: 0.2–0.65; $p < 0.001$) following a complete course of ANS.[38] However, many studies have shown variable results. Williams et al.[42] reviewed different steroid regimens for accelerating fetal lung maturation but found minimal or no difference in the risk of GM-IVH (RR: 0.71; 95% CI: 0.28–1.81; 4 RCTs, $n = 1,902$ participants; $I_2 = 62\%$, CoE: low). In another study, Walters et al.[43] reviewed repeated doses of prenatal steroids for women at risk of preterm births and reported with moderate certainty that the evidence for reduction in severe GM-IVH was equivocal (RR: 1.13, 95% CI: 0.69–1.86; 7 RCTs, $n = 5,066$ infants). Similarly, Blankenship et al.[44] detected no difference in the risk of GM-IVH in preterm, small for gestational age (SGA) infants who had received ANS (OR: 0.82; 95% CI: 0.56–1.2; 7 studies). Razak et al.[36] performed a meta-analysis of data from 9 RCTs ($n = 4368$ participants) and found a small reduction in the risk of severe GM-IVH risk with ANS (RR: 0.54; 95% CI: 0.35–0.82; $I_2 = 36\%$, aRD, –1% 95% CI, –2% to –0%); NNT 80 (95% CI: 48–232; CoE: moderate). However, there was no treatment effect for other interventions, including betamethasone vs dexamethasone for lung maturity (RR: 2.17; 95% CI: 0.89–5.25, $I_2 = 0\%$, 4 RCTs, $n = 1956$ participants, CoE: moderate), or repeated vs a single course of steroids (RR: 1.06; 95% CI: 0.73–1.56; $I_2 = 13\%$, 8 RCTs, $n = 5,472$ participants, CoE: moderate).

Magnesium Sulfate

Antenatal magnesium sulfate ($MgSO_4$) is a frequently used tocolytic medication that can temporarily stop or slow down uterine contractions to prevent preterm births. Antenatal $MgSO_4$ promotes myocardial stability in mothers, improves placental and fetal neural blood supply, reduces ischemic changes, and has antioxidant effects, especially in the brain, against apoptosis and hypoxia.[45,46] It also normalizes platelet aggregation.[47–49]

A subgroup analysis focusing on the $MgSO_4$ regimen showed that a single 4g dose of $MgSO_4$ can protect again GM-IVH (RR: 0.86, 95% CI: 0.66–1.12; $I_2 = 0\%$; $p = 0.473$).[50] Another subgroup analysis based on gestational age showed that the effect of $MgSO_4$ on GM-IVH in premature infants between 24 and 37 weeks and <34 weeks was 0.93 (95% CI: 0.83–1.05; $I_2 = 74.1\%$; $p = 0.009$) and 0.92 (95% CI: 0.7–1.18; $I_2 = 56.7\%$; $p = 0.099$), respectively.[50] The same investigators noted a significantly lower risk of severe GM-IVH (adjusted OR 0.248 (95% CI: 0.092–0.66, $p = 0.006$) in very preterm infants exposed to antenatal $MgSO_4$. Other groups have noted similar results.[49,51] However, the results have not been consistent across all studies. Razak et al.[36] did not find any effect of antenatal $MgSO_4$ on the risk of severe GM-IVH (RR: 0.8; 95% CI: 0.61–1.06; $I_2 = 10\%$; 6 RCTs, $n = 4,559$ participants; CoE: moderate). A recent meta-analysis of 7 pooled studies also did not show a significant protective effect of antenatal $MgSO_4$ on GM-IVH in preterm infants (RR: 0.8, 95% CI: 0.63–1.03; $I_2 = 63\%$; $p = 0.013$, $n = 7,236$ infants).[50]

Tocolytic Agents other than $MgSO_4$

There are various established guidelines for using tocolytic agents with mothers at high-risk for preterm labor. Most international guidelines, including those from The American College of Obstetricians and Gynecologists (ACOG), do not approve of long-term use of tocolytics because even though these medications may delay preterm births, the effects on neonatal outcomes are not consistently positive.[52,53] Some studies recorded adverse effects on infants. In a retrospective literature review on antenatal use of tocolytics and GM-IVH, Doni et al.[54] evaluated 241 very preterm infants who were exposed to antenatal indomethacin given as a tocolytic; multivariate analysis of the study showed a possible association between antenatal indomethacin and all degrees of GM-IVH (OR: 3.16; 95% CI: 1.41–7.05). Hammers et al.[55] systematically reviewed 27 observational studies with 8,454 infants (1,731: antenatal indomethacin exposure and 6,723: not exposed). The risk of severe GM-IVH (RR: 1.29; 95% CI: 1.08–1.71) and periventricular leukomalacia (RR: 1.59; 95% CI: 1.17–2.17) was higher in the antenatal indomethacin exposure group.

Pinto Cardoso et al.[56] assessed death and/or GM-IVH (primary outcome) in preterm infants between 24 and 31 weeks' gestation from the French 2011 EPIPAGE-2 cohort. Groups of preterm infants with vs without tocolytic exposure and groups with atosiban (oxytocin receptor antagonist) vs calcium channel blocker exposure were compared. Death and/or GM-IVH were not significantly different in preterm infants with vs without tocolytic exposure [183 of 363 (50.4%) vs 207 of 363 (57.0%); RR: 0.88; 95% CI: 0.77–1.01; $p = 0.07$]. The secondary outcome (death and/or grades III-IV IVH) was significantly lower in preterm infants with vs without tocolytic exposure [92 of 363 (25.3%) vs 118 of 363 (32.5%); RR: 0.78; 95% CI, 0.62–0.98; $p = 0.03$]. A secondary analysis of the APOSTEL-III trial, where 102 Dutch women were treated with nifedipine or atosiban for threatened preterm labor between 25 and 34 weeks' gestation, showed no differences in overall brain injury (abnormal cranial ultrasounds).[57] In another study, Weintraub et al.[58] reported a lower incidence of severe GM-IVH in preterm infants (24–32 weeks) exposed antenatal to ritodrine, a betamimetic tocolytic, compared to magnesium sulfate or indomethacin. Ritodrine has been removed from the American market due to adverse cardiovascular effects, but is occasionally still used as a tocolytic internationally.[59–61]

Delivery and Stabilization

Type of delivery: Korcek et al.[38] showed a borderline reduction in GM-IVH following a Cesarean section (CS; OR: 1.85; 95% CI: 0.98–3.51, $p = 0.057$). Other studies have also indicated that elective CS protects against GM-IVH in preterm infants, and perhaps antenatal steroids in combination with CS might have further enhanced this protection in extremely preterm infants born between 24 and 25 weeks gestation.[42,57,62–64] The ACOG suggested CS might be considered and recommended for preterm infants at a gestational age of 23–24 weeks and 25 weeks, respectively. Similarly, Karayel Eroglu et al.[65] reported lower rates of GM-IVH in preterm infants <34 weeks' gestation following CS. Humberg et al. from the

German Neonatal Network also showed elective CS reduces the risk of GM-IVH in infants <30 weeks' gestation.[66] Huang et al.[67] studied 826 Taiwanese infants and found a similar reduction in the risk of GM-IVH in CS-delivered infants between 22 and 25^{+6} weeks' gestation.

The benefits are not so clearly discernible at 2 years of corrected age. In two studies, Rahman et al.[68] and Ljustina et al.[69] did not detect a notable effect of CS on the incidence of GM-IVH in preterm infants born at the gestational age of 27–$34^{+0/7}$ weeks. Luca et al.[70] also did not find any association between the delivery mode and

Delayed cord clamping (DCC): Delayed cord clamping can be an important adjunct to the GM-IVH bundle.[32,71] The ACOG recommends DCC in all preterm neonates for at least 30–60 seconds.[72] A Cochrane review analysis reported that compared to early cord clamping (ECC), DCC decreases any GM-IVH with high-certainty evidence (RR: 0.83; 95% CI: 0.70–0.99); mortality with moderate-certainty evidence; and severe GM-IVH and periventricular leukomalacia (PVL) with low-certainty evidence.[73] Many other studies support a similar protective effect of DCC against GM-IVH in preterm neonates.[74–76] In utero, oxygenated blood from the placenta passes through the umbilical vein to the right atrium. However, pulmonary vasoconstriction can cause this blood to bypass the lungs and flow directly across the foramen ovale and the ductus arteriosus to the systemic circulation. After the onset of breathing, atmospheric oxygen is delivered to the pulmonary vascular bed, with consequent pulmonary vasodilation. It increases right ventricular output, leading to an upsurge in blood flow from the low-resistance placenta to the neonate.[77] Early cord clamping with immature respiratory drive can compromise left ventricular preload. There might be a two-edged effect: (A) a substantial amount of blood remains within the placenta after birth, and (B) pulmonary vasoconstriction and consequently restricted pulmonary blood flow further limits left ventricle filling. Early cord clamping is defined as clamping the umbilical cord <30 seconds after birth. There is a lower total blood volume and, thus, an increased likelihood of neonatal anemia and risk of intestinal injury, as well as GM-IVH.[78,79] Delayed cord clamping can benefit by reducing IVH. The delay in cord clamping has been traditionally defined as >30 seconds, but a longer duration of ≥60 seconds, until the cord pulsations fade, might be even more effective (level of evidence: 1a).[73,80,81] Delayed cord clamping adds about 5–20 mL/kg blood from the placenta into the newborn circulation, and consequently, lengthens the time for postnatal reduction in pulmonary vascular resistance.[82,83] Delayed cord clamping might be protective due to improved cerebral vascular autoregulation and fewer fluctuations in systemic perfusion.[83] In most studies, there is information on the placement of the infant's head vis-à-vis the mother's bed; the infant's head has been positioned with gravity to enhance flow to the infant. There are concerns that head elevation could adversely affect cerebral blood flow due to low umbilical venous pressures.[62] However, none of the trials have shown whether a combination with subsequent midline head positioning could have added benefits.[84]

A recent Cochrane review of 48 studies involving 5,721 babies and their mothers analyzed data from 40 studies involving 4,884 babies and their mothers. Infants born at 24–36^{+6} weeks› gestation and multiple births were included. Delayed cord clamping was seen to have slightly reduced the number of infants with any Grade IVH: Average risk ratio (aRR) (0.83, 95% CI: 0.7–0.99, 15 studies, 2,333 babies, high certainty) but not alter the number with severe IVH (grades III and IV) (aRR 0.94, 95% CI: 0.63–1.39, 10 studies, 2,058 babies, low certainty).[73] In most studies, the need to discontinue DCC originated from maternal/neonatal instability, fetal anomalies such as diaphragmatic hernia, disrupted placental circulation, monochorionic twins, intrauterine growth restriction, and hydrops fetalis.[78,85,86] A study of 474 infants in 2019 raised concerns about a high incidence of IVH associated with umbilical cord milking. It was stopped early when more IVHs were seen for preterm infants (23–27 weeks' gestation) than in similar infants in the DCC group.[62] Hence, cord milking is not recommended in preterm infants < 28 weeks' gestation. Globally, most centers lean towards trying DCC for infants born at ≥ 32 weeks' gestation.

Table 1: Main components of the "Golden Hour" strategy

1	Antenatal counseling, team briefing, and debriefing
2	Delayed cord clamping
3	Prevention of hypothermia (maintain temperature 36.5–37.5°C)
4	Timely and appropriate respiratory support
5	Cardiovascular system support
6	Nutritional support to prevent hypoglycemia
7	Infection control
8	Laboratory investigations to provide minimal handling afterwards
9	Monitoring and record keeping.
10	Family counseling

Golden-hour Management

Preterm infants are often ill-equipped for spontaneous transition to extrauterine life. Many require resuscitative help in the delivery room. There is sufficient evidence to support that preterm neonates should receive timely delivery room CPAP for better respiratory stabilization in the form of a decreased need for surfactant and mechanical ventilation.[87] However, this need for delivery room support can be an independent risk factor for GM-IVH in preterm neonates.[88] A retrospective study from Japan further showed that delivery room intubation was associated with an increased risk of severe GM-IVH.[89] The Golden Hour strategy is an evidence-based, standardized, structured care program supporting fetal-neonatal transition. This approach can be important for reducing the incidence of GM-IVH as nearly 50% of GM-IVH cases occur within the first 24 hours of birth, which can be altered with proper resuscitation in the delivery room and stabilization in the Neonatal Intensive Care Units.[90] Preventive measures should be implemented before this critical time frame. Golden Hour management has improved many short- and long-term adverse outcomes in extremely premature ELBW infants.[91–97] Short-term complications may include hypothermia, hypoglycemia, and respiratory and cardiovascular instability, which seem relatively benign but can increase the risk of GM-IVH. Most complications may be preventable with proper interventions during this crucial period. Indeed, there is some evidence that the Golden Hour strategy can significantly reduce the incidence of GM-IVH (level of evidence: 1a) (Table 1).[91,98]

Gentle Care

Transportation: There is a need for cautious transportation of at-risk premature infants to prevent GM-IVH. Outborn neonates, born in facilities without an appropriate-level NICU, experience logistical challenges during transfer to tertiary perinatal centers. Data show that preterm infants delivered in tertiary perinatal

centers have better outcomes, including fewer GM-IVHs, than those born in less-equipped hospitals and then transferred to tertiary centers for subsequent management.[99,100] These data were further supported in a large retrospective multicenter study of nearly 67,600 VLBW infants, where those who were transported within the first 48 hours after birth showed a higher frequency and severity of GM-IVHs.[101] Similarly, Towers et al.[102] showed a higher risk for significant GM-IVH (Grade III or IV) in VLBW infants born in level 1 nurseries and transported to higher centers than those born at their level 3 facility. Transport of preterm infants between facilities is believed to be an independent risk factor for GM-IVH and acute brain injury.[103,104] In one study, the incidence of GM-IVH was 27.4% in transported infants, compared to 13.42% in non-transported infants. The adjusted OR was 1.75 (95% CI: 1.64–1.86, $p < 0.001$).[100] The relationship between the transportation of VLBW newborns between hospitals and their susceptibility to GM-IVH is not completely understood and is possibly multifactorial. Vigorous manipulations, kinking or obstruction of the endotracheal tube, accidental extubation, or iatrogenic trauma while moving the infant. Exposure to low body temperature and unstable temperature during transportation can negatively affect blood flow to organs, leading to lactic acidosis, and possible delay in providing nutrition that may lead to hypoglycemia. This condition may be linked to GM-IVH; however, this correlation is not definitive.[100]

Hypothermia and temperature instability during transport could compromise organ perfusion and cause lactic acidosis, increasing the risk of GM-IVH.[102,105,106] The presence of transport hazards such as noise, vibration, acceleration and deceleration forces, additional handling, temperature fluctuations, and space and movement limitations, along with the need to address clinical deterioration, highlight the importance of creating, implementing, or strengthening a GM-IVH bundle in pre-transport management.[107]

Several steps can be useful components for preventing neurological injury in VLBW infants during transport in hospitals that handle the delivery before the transportation of VLBW newborns and in hospitals that offer interhospital transport for additional care. If there are no maternal risks or imminent delivery, *in-utero* transport to a hospital with an appropriate tertiary care NICU improves the overall outcomes in preterm neonates.[108] Similarly, telemedicine effectively expands access to quaternary neonatal care for rural communities and level I and level II NICUs, reduces potentially-avoidable transfers of newborns to level III and IV NICUs, helps in the triage of neonatal transfers, promotes family-centered care and reduces healthcare costs.[109,110]

Implementation of a prevention bundle for hypothermia has been shown to reduce the incidence of GM-IVH.[111] Placing polyethylene bags before cord clamping as an isolated intervention has not changed the proportion of neonates with normothermia.[112,113] Existing data show that maintaining normothermia (36.5–37.5°C) during and after the golden hour in all neonates born <34 weeks' gestation can help prevent hypoxic-ischemic encephalopathy (HIE). Using snap-open access port covers or air-boosts on transport incubators reduces hypothermia.[114] Use of gel-filled thermostable mattresses, cots with water-filled mattresses, maintaining delivery room temperatures at >25°C, reducing maternal hypothermia before delivery, providing plastic bags/wraps, and caps for the newly born infants, using warm resuscitation gases, room humidity optimized for the country, season, and gestation may decrease hypothermia at NICU admission and be associated with a lower risk of severe brain injury and mortality.[115–123] Double-walled whole-body polyethylene bags, either as an isolated step or in combination with an exothermic mattress, can increase the risk of hyperthermia and preterm brain injury.[124–128] Surface temperature (axillary) may be sensitive to hypothermia but not hyperthermia.[113] Non-invasive temperature measurements using non-contact infrared thermometers (NCITs) and infrared thermographic (IRT) have shown a reasonable accuracy of ± 0.3°C in various settings but have not been evaluated during transport. The performance of these devices has been influenced by measurement location, the type of sensor, the reference and tool, individual physiological attributes, and the surrounding environmental conditions.[129]

Noise and vibration could be important variables in the causation of GM-IVH, particularly during transfer. The cerebral vasculature in premature infants is still not fully developed in structure and has also not matured with autoregulation. Indeed, noise and vibration have been associated with fluctuations in blood pressure, heart rate, and respiratory rates, leading to variability in cerebral blood flow and consequent risk of GM-IVH.[130–133] The American academy of pediatrics has (AAP) recommended that sound levels in the NICU should not exceed 45 dB. The International Electrotechnical Commission (IEC) recommends standard maximum sound levels of <60 dBA for transport incubators and vibration levels of < 0.31 m/s^2.[24,134] Having GM-IVH bundles with ongoing staff education programs and staff awareness of monitoring noise and sound levels that reach the neonate during transport can diminish the effects of stress on the babies' cardiovascular, respiratory, neurological, and endocrine systems and reduce adverse neonatal outcomes. The current sound levels in the transport incubator range from 47 to 55 dB. In an observational study, peak sound levels ranged from 80.4 to 86.4 dBA in rotor wing air transport (RWAT) with whole-body vibrations (WBV) of 1.68–5.09m/s^2. In ground ambulance transport (GAT), the noise was 70.3–71.6 dBA and WBV 1.82–3.96m/s^2.[111] Silicone ear plugs or earmuffs can reduce sound level exposure, and gel mattresses can lower the vibration in air transport. These benefits from vibrations were not evident in GAT.[135] Early encouraging data suggest that non-contact active noise cancellation (ANC) devices deserve further testing.[136] Dedicated Neonatal transport service and newborn emergency transport service (NETS) teams can reduce transport-related morbidity and mortality.[137,138] These teams can be comprised of registered nurses, respiratory therapists, physicians, and paramedical staff.[139] They can help stabilize the infant prior to transfer, including with delivery room resuscitation at the peripheral centers.[140] Thus, although inter-hospital transport increases the risk of GM-IVH, careful training and preparation, including experienced personnel, and strict adherence to guidelines can help. The goal is to ensure these vulnerable newborns receive the highest standard of care while minimizing potential harm and improving their outcomes.

Neonatal Intensive Care Unit (NICU) Management

Nursing Care

Head positioning: Optimizing care practices to ensure adequacy and less variability in the cerebral blood flow of preterm infants is crucial in the first 72 hours after delivery. Maintaining a neutral head position and minimizing head tilt could prevent jugular venous obstruction and subsequent ipsilateral venous congestion.[141,142] Early in the literature review, from 1980 to 2010, 10 articles were clinical trials, providing either type III evidence using a quasi-experimental, non-randomized convenience sample design or, in expert opinion, reporting an increased cerebral blood volume or

decreased jugular venous flow with head position/tilting. These explain the changes in cerebral oxygenation (increased cerebral blood velocity and/or increased intracranial pressure), with the head position as likely to be secondary to the occlusion of the jugular venous drainage, which could increase the risk of GM-IVH in premature infants (low certainty of evidence). Another observational discussion by the investigators was the connection between head tilting and brain hemodynamics. They concluded that an increased cerebral blood volume and/or intracranial pressure may result from the infants' potential inability to autoregulate cerebral blood flow adequately.[143]

A Cochrane review in September 2019 identified 3 RCTs with a total of 290 infants (either < 30 weeks' gestational age or < 1000 gm body weight) on the effects of head midline position on GM-IVH in very preterm infants. Two of them compared the midline position to the head rotated at 90° with the flat cot. The third trial compared the supine midline head position with the head rotated 90° and the bed tilted at 30°. The result of this trial did not show any effect on rates of GM-IVH with RR: 1.11, 95% CI: 0.78–1.56, and severe GM-IVH with RR: 0.71, 95% CI: 0.37–1.33. Neonatal mortality (RR: 0.49, 95% CI: 0.25–0.93) was lower in the supine midline head position.[144] However, the certainty of the evidence was very low for all outcomes, primarily due to the limitations of the study design. High-quality RCTs are needed to resolve this uncertainty. Studies have also shown that when we support infants in a tucked flexed position, with hands to the midline or near their face, we enhance autonomic stability and promote relaxation, which, in turn, can be beneficial for premature infants by promoting self-regulation, maximizing stability, optimal breathing patterns, reserving energy, and fostering overall growth and development.[143,145] After benchmarking NICU centers with low GM-IVH rates were reported to the Vermont Oxford Network and reviewed, the experts identified midline positioning aligned with the torso and 30º bed elevation as potential best practices, but without proven evidence. This practice is essential to avoid fluctuations in intracranial pressure that may increase the risk of acute brain injury.[142]

Handling care: Nurses play a critical role in interventions in the first 72 hours of life, a period when preterm infants are most susceptible to GM-IVH.[146,147] As stated earlier, the nurse should maintain the infant in a comfortable, midline, flexed, and contained position with the infant's limbs flexed and tucked using appropriate positioning aids and boundaries.[147,148] Maintaining the hands near the face and gently repositioning the baby after extending their limbs during exams and procedures is another IVH prevention nursing measure.[149] For handling preterm and sick babies, the nurses should gently handle the babies with slow, controlled movements. They should act as a team and seek assistance from additional nurses for procedures or complex handling maneuvers.[141,143] All these recommendations are based on potential best practices. It has been advised to avoid routine suctioning for preterm newborns unless there are objective reasons to warrant it.[150]

Blood withdrawal: Another nursing element that warrants careful attention is avoiding rapid blood withdrawal of blood from the umbilical arterial line. This practice is associated with a transient but significant drop in cerebral blood flow. Previous studies have noted low cerebral tissue oxygen saturations ($CrSO_2$s) and increased $CrSO_2$ variability in real-time NIRS monitoring during umbilical arterial blood sampling. The assumption here is that temporary changes in cerebral blood flow led to increased cerebral tissue oxygen extraction. Whether patient or procedure-related factors are the primary contributors to this phenomenon remains unclear.[64,151]

The impact of the sleep-wake cycle on the risk of IVH is plausible yet unproven. Sleep is crucial to brain development, especially for 22–36 weeks-old gestation premature infants.[152] Sleep patterns begin to mature around 28 weeks' gestation, with most of the sleep time being in REM sleep.[153] The percentage of REM decreases with development. This critical period allows adequate neuronal maturation, ranging from neuronal differentiation, migration, and myelination to synapse formation and elimination to central visual development and relationships with the other sensory systems.[154] Recent literature suggests better practices for preserving and promoting infant sleep within the NICU.[152] It is well recognized that the sleep-wake cycle directly impacts brain development and future learning and is associated with improved long-term developmental outcomes.[152] While term infants sleep around 70% of the day, expected preemies sleep even more. However, more studies are required to determine the exact sleep requirement for developing the preterm brain. In 2005, Holditch-Davis et al. assessed sleep-wake states in 71 preterm infants. Evaluation occurred over 3 years, with 51 enrolled looking at IQ, motor development, and the home environment. The preterm infants that developed REM sleep more rapidly showed the best outcomes (Mean et al. scores, language development, and fine motor skills).[155] Based on available data, assessing the infant's sleep-wake cycle is essential to evaluating the appropriate timing for positioning and caring. Minimizing disruptions, repositioning the infant gently and carefully at fixed intervals, and aligning care activities with the infant's sleep-wake states whenever possible played a positive role.[64]

To reduce the stress on critically-ill infants, the nurses should use minimal handling techniques and suction the infants very gently and infrequently.[150] Routine endotracheal suction should be avoided. These protocols reduce stress and hemodynamic fluctuations. This nursing care, again, is based on best practices (level of evidence: 5).

Respiratory Management

Surfactant therapy could reduce the risk of GM-IVH. The relation between the use of surfactants and improved preterm survival has been well established.[156] Until recently, one protocol for surfactant delivery involved Intubation, Surfactant delivery, and Extubation (InSurE) followed by support with a continuous positive airway pressure device. However, in view of the requirement of intubation and bag and mask ventilation in this technique, alternative, less invasive approaches have been evaluated. Surfactant instillation via a laryngeal mask, aerosolization, and thinner catheters have all been evaluated. Using a thin or more flexible catheter for surfactant delivery appears to have been associated with enhancing neonatal outcomes, including a decrease in the need for mechanical ventilation in the first 72 hours.[157] A recent Cochrane review of 16 studies and 2,164 neonates concluded that less invasive surfactant administration (LISA) with surfactant administration with ETT significantly reduced the need for intubation within 72 hours (RR: 0.63, 95% CI: 0.54–0.74), risk of the composite outcome of death or BPD at 36 weeks' postmenstrual age (RR: 0.59, 95% CI: 0.48–0.73), severe GM-IVH (RR: 0.63, 95% CI: 0.42–0.96), death during first hospitalization (RR: 0.63, 95% CI: 0.47–0.84), and BPD among survivors (RR: 0.57, 95% CI: 0.45–0.74).[158] Techniques such as surfactant administration through the laryngeal or supraglottic airway (SALSA) and aerosolized surfactants are

currently being investigated.[159] Surfactant therapy and mechanical ventilation have reduced the mortality related to RDS, but there could have been increased rates of GM-IVH. Further studies are needed to explore these associations.[160] Increasing data suggest that non-invasive respiratory support can improve outcomes over intubation and mechanical ventilation, even though some infants will need lifesaving assisted ventilation at birth or during a NICU stay.[161,162] The risk of intubation and mechanical ventilation increases with decreasing gestation, as does the risk of GM-IVH. A recent Cochrane review of 20 RCTs showed that volume guarantee ventilation lowered the risk of Grade 3 or 4 GM-IVH compared to pressure-limited mode.[163]

The effects of high-frequency oscillatory ventilation (HFOV) on GM-IVH and other neurological outcomes remain controversial and inconclusive. Some RCTs have shown that HFOV could increase the risk of all grades of GM-IVH, but the results were not consistent.[164,165] At 2 years of age, the outcomes did not clearly differ between the two groups.[166] There is a need for further study with appropriately sized samples.

Oxygenation: Current evidence suggests using blenders in the delivery room with FiO_2 of 30% for <28 weeks and 21–30% for those born between 28 and 31 weeks.[167,168] In addition to promptly starting SpO_2 monitoring after birth, the recommendation is to modulate FiO_2 to achieve SpO_2 >80% by 5 minutes after birth, since there is a demonstrated association between hypoxia at this time point and increased mortality and GM-IVH.[169] The immature GM is very fragile and prone to hyperoxia-induced free oxygen radical injury as well as hypoxia. For this reason, several RCTs and meta-analyses have been conducted to define "the perfect" oxygen saturation in neonates.[170,171] To balance the risks of retinopathy vs injury to other organs, oxygen concentrations may need careful titration based on gestation and birth weight.

Permissive hypercapnia: A key goal of respiratory assistance is to achieve adequate ventilation (CO_2 elimination). Hypocapnia ($PaCO_2$ <35 mm Hg) is associated with cerebral vasoconstriction and associated white matter injury, whereas hypercapnia is linked to increased cerebral blood flow with a subsequent increased risk of GM-IVH.[172] Though experimental studies showed a beneficial effect of higher $PaCO_2$ on the lung, studies in ventilated preterm neonates showed no clinical benefits.[173] Currently, there are no safe thresholds to define permissive hypercapnia. Severe hypercapnia is associated with an increased risk of GM-IVH. Apart from this, fluctuations in $PaCO_2$ levels have been found to have a more deleterious effect on the severity of GM-IVH.[174] Avoiding $PaCO_2$ levels of >60 mm Hg during the first 72 hours of life in preterm neonates is recommended.[175] Assisted ventilation should be provided to optimize gas exchange while minimizing lung injury and its associated morbidities.[174]

Hemodynamic Management

Treating hypotension: There is no consistent definition of hypotension or a standardized approach to its management in preterm infants.[148] Blood pressure levels <5th percentile for gestational and postnatal age are considered concerning.[176] Lightburn et al.[148] found no significant difference in cerebral blood flow velocities between ELBW infants with and without documented hypotension. However, other studies have linked the use of vasopressors for treating hypotension in preterm infants to an increased risk of developing GM-IVH and other brain injuries.[177,178]

A Cochrane review of 8 RCTs did not find evidence to support the routine use of volume expansion in preterm infants without cardiovascular issues or in infants with cardiovascular compromise when outcomes such as severe disability, cerebral palsy, or mortality were reviewed.[179] A cautious approach is recommended; a slowly infused fluid bolus may be useful before initiating inotropes. Inotropes should be considered when low blood pressure is associated with a prolonged capillary refill, decreased urine output, elevated lactate, or abnormal echocardiography findings (grade B recommendation).[180]

Management of a patent ductus arteriosus (PDA): Patent ductus arteriosus is a common morbidity of the preterm infants. The presence between PDA has been linked to various morbidities in neonates, including intraventricular hemorrhage.[181,182] The association of PDA and GM-IVH is complex. Although many studies suggest that the presence of hemodynamically significant PDA has been associated with GM-IVH.[183,184] Many other studies have contradictory results that negate its considerable direct effect on cerebral circulation.[180,185] Patent ductus arteriosus and its relation to GM-IVH is a complex interplay of various factors between the susceptible host, hypoperfusion, hemodynamic changes by PDA, coagulation, and subsequent exposure to reperfusion and pharmacological agents used to treat PDA.

Hemodynamic changes due to PDA: Cerebrovascular autoregulation plays an important role in maintaining adequate cerebral perfusion. Numerous studies have demonstrated that premature infants have impaired cerebrovascular autoregulation.[186,187] The association of hemodynamically significant PDA with GM-IVH needs further study. Significant PDA in the presence of cardiac immaturity and increased cerebrovascular reactivity could contribute to GM-IVH. Significant controversy prevails around the management of PDA, and several care pathways are being followed, including conservative management, aggressive treatment, targeted treatment, or surgical ligation. The outcomes need to be carefully studied.

Conservative approach in PDA management: This is a highly-debated approach to managing a hemodynamically significant PDA. Sung et al.[188] compared two epochs. Epoch I (July 2009–Dec 2011) and Epoch II (Jan 2012–Jan 2014) in the management of PDA in ELBW infants, comparing a nonintervention approach vs a mandatory closure approach in infants with a PDA >2 mm. In Epoch I, PDA was managed with indomethacin followed by surgical ligation, and nonintervention was used in Epoch II. There were no significant differences in mortality or morbidities like necrotizing enterocolitis or intracranial hemorrhage (ICH). Interestingly, in Epoch II, despite longer exposure to PDA, rates of BPD were lower. During the same period, another study in the US that included over 5,000 VLBW infants showed contradictory findings suggesting that while the rate of PDA intervention decreased, there was an increase in morbidities like bronchopulmonary dysplasia (BPD), periventricular leukomalacia (PVL), retinopathy of prematurity (ROP), and acute renal failure.[189] Hence, extrapolating these findings to various populations and centers is difficult and requires further investigation.

Prophylactic closure: Early prophylactic indomethacin, if it started earlier, between 6 and 12 hours of life, and continued every 24 hours for a total of 3 doses, has been shown to decrease the incidence of any GM-IVH (RR: 0.88, 95% CI: 0.8–0.98), and severe GM-IVH (RR: 0.66, 95% CI: 0.53–0.82) in extremely low birth weight neonates.

It decreased the incidence of hemodynamically significant PDA and the need for surgical ligation. Still, it did not change motor/sensory outcomes at 36 months of corrected age.[190,191] However, a large case-control study of 633 infants showed a higher risk of spontaneous bowel perforation when babies were exposed to indomethacin during the first three days after birth compared with control infants [odds ratio (OR) 1.86, $p < 0.0001$].[192] Also, a retrospective analysis of the ELBW cohort in the Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network showed similar results when it was used for PDA closure with a therapeutic, not prophylactic intent (adjusted OR: 1.61, 95% CI: 1.25–2.08, $p < 0.05$).[193] However, no randomized control trials comparing prophylactic indomethacin to placebo (including a Cochrane meta-analysis) have shown an increased risk of SIP or NEC.[194] The incidence of severe GM-IVH was dramatically decreased in infants born at <29 weeks who had not received any antenatal steroids.[195] There could be a possible therapeutic window to treat <29 weeks' gestation premature infants with indomethacin who had received no or only partial antenatal steroids and had no sonographic evidence of GM-IVH.[196] On the other hand, prophylactic ibuprofen treatment did not show any reduction in GM-IVH. A 2011 Cochrane review indicated that ibuprofen prophylactic treatment did not significantly reduce GM-IVH (all grades or severity).[197] There is not much evidence on prophylactic acetaminophen and its effect on GM-IVH. Two randomized controlled trials with 80 infants indicate no appreciable improvement in patient-important clinical outcomes.[198]

Pain Management and GM-IVH

Newborns can detect, process, and respond to painful stimuli. Preterm infants may not only be more sensitive to pain due to immature inhibitory mechanisms, but they might also be exposed more frequently to painful procedures such as heel sticks/venipuncture for blood sampling.[199] In a large prospective multicenter trial in level III NICUs in Paris, neonates experienced an average of 115 (range, 4–613) procedures during the 6-week study period, with an average of 16 (range, 0–62) procedures per day of hospitalization.[200] There was clear evidence that pain and stress affect brain development adversely.[201] In rat pups, repetitive exposure to painful stimuli accentuated neuronal excitation and apoptosis in several cortical and subcortical areas, suggesting that pain and pain-related effects may have a widespread impact on the developing brain.[181] Pain-related stress during the early neonatal period can be associated with reduced white matter and subcortical grey matter maturation. Consistent findings can be noted in magnetic resonance (MR) imaging, 3D MR spectroscopic imaging (MRSI), and diffusion tensor imaging (DTI).[202] Many centers are now using near infrared spectroscopy (NIRS) to measure cerebral blood flow and oxygenation in the brain. There are clear differences in the NIRS signatures of pain-induced prefrontal activity vs. those induced by various emotional and stress responses used.[202] It is still unclear whether there is a direct causal relationship between pain and GM-IVH, but there is clear evidence that pain leads to stress-related neurodevelopmental changes. Therefore, pain-control measures should preferably be included in GM-IVH bundles.

Skin-to-skin contact, commonly known as "Kangaroo mother care" (KMC), is vital in providing comfort to neonates when allowed and is one of the primary methods of non-pharmacologic intervention for pain management.[203] Several studies are currently investigating the impact of KMC on the occurrence of GM-IVH. For KMC, infants are placed in a prone position on the parents' bare chest, enabling direct contact between the neonate and the parent.[204] Various research studies have shown the positive effects of KMC on easing the discomfort of neonates and significantly improving their overall outcomes. Kangaroo mother care strengthens mother-infant sensory interaction and induces hormonal and epigenetic processes to enhance preterm infant health.[29] It also mitigates pain, promotes bonding with parents, and increases breast milk supply. A study reported decreased mortality and improved long-term developmental outcomes with KMC. The relationship between KMC and GM-IVH is being investigated with interest. The British Association of Perinatal Medicine and Neonatal Society has implemented "side-lying KMC while maintaining midline position" to improve overall outcomes, including GM-IVH and pain control.[205] Collados-Gómez et al.[206] are conducting a trial to evaluate side-lying positions for hemodynamically stable <28 weeks' gestation neonates for various outcomes, including GM-IVH.[206] Although there is still limited evidence of utilizing KMC in premature neonates to reduce GM-IVH and improve pain control, studies are already showing the positive effects of KMC in easing the discomfort of neonates.[206] Similarly, early results suggest that swaddling to simulate the *in-utero* position in neonates might also enhance the level of comfort.[207] In neonates receiving some oral feeds, oral sucrose gel is another non-pharmacological intervention that has proven beneficial. It is a simple yet very effective measure for frequently-performed procedures such as heel sticks or venipunctures.[187] In short, KMC, swaddling, and oral sucrose are robust non-pharmacological interventions that enhance neonatal care for premature neonates and promote healthy neurodevelopmental care.

Noise Reduction and Controlled Environment

Many units have formed task forces/champions to alleviate noise by maintaining noise levels under 65–70 decibels (dB). In conjunction with maintaining noise level under 65 dB, placing noise reduction posters across the unit, rounding on patients away from their isolette, and adjusting the overall announcement and paging system to a lower decibel level have made it successful in maintaining NICUs to minimal stimulation units. Although there might not be a demonstrated direct relationship with IVH, low noise levels seem to be neuroprotective.[208]

Neonatal Thrombocytopenia and Platelet transfusion: A Complex Interplay

Neonatal thrombocytopenia: Studies have been reported that fetal/neonatal thrombocytopenia can increase the risk of IVH.[209] In a prospective study conducted by Kahn et al.[210] on VLBW infants, they reported that IVH Grade >2 was noted in 20.7% of infants with platelet counts <100,000/μL, which was significantly higher than 6.4% of those without thrombocytopenia. Some other studies have also shown that the risk of GM-IVH increases with the severity of thrombocytopenia.[211,212]

The association between thrombocytopenia and GM-IVH has not been consistent across all studies of critically-ill premature infants. Bolat et al.[190] focused on neonates in the NICU with thrombocyte counts $<50 \times 10^9$/L and found a higher prevalence of IVH ≥ Grade II in infants with thrombocytopenia (7.2%) than in those without thrombocytopenia (4.4%). However, these findings

were not significantly related to the level of the lowest platelet count. In a retrospective multicenter cohort study, Sparger et al. observed thrombocytopenia as a risk factor for IVH, but the severity of thrombocytopenia was not correlated with GM-IVH.[213] Von Lindern et al.[214] found that GM-IVH Grade ≥ II was higher in neonates with thrombocytopenia. However, after conducting a multivariate linear regression analysis, the risk in the subgroups of thrombocytopenic infants was not significantly different ($p = 0.3$). A recent systematic review of 6 studies summarized insufficient evidence for a causal relationship between platelet counts and the risk of GM-IVH in neonates.[215] The authors inferred that further study is needed to explore the possibility of confounding variables such as lower gestational age, intensity of illness, low birth weight, or sepsis. In some studies, IVH was discovered before the onset of thrombocytopenia, implying that the low platelet counts might not have been the direct cause.[214]

The importance of thrombocytopenia as a causative variable in GM-IVH is evident in studies of fetal/neonatal alloimmune thrombocytopenia (FNAIT). In this condition, there are fewer confounding factors. An observational cohort study of ICH caused by FNAIT from the International No IntraCranial Hemorrhage (NOICH) registry during the period 2001–2010 (13 tertiary referral centers from 9 countries across the world) examined 592 cases of FNAIT in the registry, and 43 confirmed cases of ICH due to FNAIT were noted. Most hemorrhages (23/43, 54%) occurred before 28 weeks' gestation.[216] Intracranial hemorrhage due to FNAIT is reported to occur in 1:12,500–25,000 births; it has been recorded more frequently at full-term birth as isolated ICH or its consequent clinical features, such as seizures, might not be noticeable in utero.[216] Fetal/neonatal alloimmune thrombocytopenia is caused by maternal alloantibodies directed against fetal platelets due to their incompatibility with human platelet antigens (HPAs).[217] Human platelet antigens -1a is a frequently incriminated antigen; the presence of HLA-DRB3*01:01 and HLA-DRB4*01:01P (*01:01 or *01:03) are important predictors of this isoimmunization.[218] Besides HPA-1a, HPA-5b is another possible antigen that may lead to FNAIT.[217]

Radder et al.[219] described 62 pregnancies among 27 mothers. They looked for the risk of ICH in successive pregnancies with thrombocytopenia, with or without a history of ICH. In 52% of the ICH cases, a previous sibling suffered from ICH. The recurrence rate of ICH in the subsequent offspring of women with a history of FNAIT with ICH was 72% (95% CI: 46–98%). Delbos et al.[220] showed that FNAIT-related ICH was associated with death in 59% of cases. In another study in the FNAIT registry, 389 people were studied; they were from Australia ($n = 74$), Norway ($n = 56$), Slovenia ($n = 19$), Spain ($n = 55$), Sweden ($n = 31$), the Netherlands ($n = 138$), and the USA ($n = 16$).[221] The median follow-up was 5 days (interquartile range, IQR 2–9). Severe thrombocytopenia (platelet count $<50 \times 10^9$/L) was reported in 283 (74%), and extreme thrombocytopenia ($<10 \times 10^9$/L) was reported in 92 (24%) neonates. Severe ICH was noted in 22 neonates.

Coagulation mediators: In addition to thrombocytopenia, we need further examination of coagulation mediators such as vitamin K reductase complex, apolipoprotein E (APOE)2, APOE4, endothelial nitric oxide synthase (eNOS *894G > T* and *–786T > C*), fibronectin 1, factor V Leiden mutation (Arg506Gln), and collagen 4A1 gene; these may play a role in GM-IVH development.[222–228] These mutations have also been implicated in cerebral hemorrhages.

Platelet transfusion threshold: The thresholds for platelet transfusions in neonatal thrombocytopenia have increasingly become more restrictive.[229–231] Andrew et al.[230] performed an RCT on thrombocytopenic ($50–150 \times 10^9$/L) preterm infants and found no difference in the incidence of bleeding between groups with and without platelet transfusion; this indicates that liberal PT might be unnecessary. In another retrospective study comparing liberal and restricted PT approaches in premature infants, no significant difference in hemorrhagic events was found between the groups.[214] Moreover, The PlaNeT-2 trial concluded that administering platelet transfusion at a lower threshold (below 25×10^9/L) resulted in lower mortality rates and fewer hemorrhage episodes than a higher threshold (PC below 50×10^9/L).[229] Several studies have shown fewer adverse effects in restrictive groups who received less platelet transfusion.[232] Kumar et al.[233] and Kasap et al.[234] also found that mortality and the GM-IVH rates were higher in the liberal transfusion group. There is a possibility that these findings might relate to the infants' clinical conditions or the properties of the transfused platelets, as those were obtained from adult donors whose platelets are hyperreactive and pro-inflammatory compared to those from infants. The actual protective value of platelets in the recipient thrombocytopenic neonates (placental counts $<20 \times 10^9$/L) is unknown. Based on current evidence, Curley et al.[229] and Kumar et al.[233] recommend relatively restrictive platelet transfusion thresholds. Since platelet transfusions can cause/augment systemic inflammatory responses, further studies are needed to determine safe, lowest-possible thresholds for platelet transfusions.[94,235]

Staff Education and Training

Continuing education in NICUs for providers and nursing staff can help promote the best, evidence-based practices to mitigate factors contributing to GM-IVH.[236] Application of strategies such as midline positing, Gentle handling, cluster care, and pain management soon after delivery could possibly help reduce GM-IVH.[237] Intermittent simulation training can also complement these ongoing education efforts.[237,238] These efforts can enhance the confidence and skills of the team, ultimately improving the quality of neonatal care and reducing the incidence of GM-IVH.[237] Frequently updated physician and nursing care guidelines can also be helpful.[152]

Other Neuroprotection Measures

Neurorestorative therapies: Multipotential stem cells, immunomodulation, and anti-inflammatory therapies have the potential to stall the progression of injury or improve neurodevelopmental outcomes.[239] Mesenchymal stem cells have been tried via intravenous and intraventricular routes, and both are safe. Prophylactic recombinant human erythropoietin therapy in very preterm infants improved cognitive outcomes at 18–24 months, without any effect on any other neurodevelopmental outcomes.[240] Low-dose melatonin (N-acetyl-5-methoxytryptamine) has benefited preterm sheep models.[241] Caffeine citrate (methylxanthine), azithromycin, and anakinra (Interleukin-1 receptor antagonist) are the other anti-inflammatory agents being studied to improve preterm brain injury.[242] Preterm neonates have high rates of clinical and subclinical seizures.[243] Magnesium sulfate infusions could help treat neurological injuries.[244] Similarly, haptoglobin levels are upregulated in the brain by oligodendrocytes after GM-IVH, and they act as scavengers of free hemoglobin and prevent free

hemoglobin-mediated cytotoxic injury.[245,246] Experimental animal studies have shown that haptoglobin infusion into the ventricular cavity may help control the adverse effects of GM-IVH.[246] However, many of the routinely used anticonvulsants have varying levels of neurotoxicity.[242]

Future Direction: Applying near-infrared spectroscopy (NIRS)

Near-infrared spectroscopy is a non-invasive tissue oxygenation and blood flow monitoring technology. It may have a potential preventable role in the incidence of GM-IVH in preterm infants. The principle of NIRS is to assess local changes in oxygenated and deoxygenated hemoglobin concentration in a body tissue region using near-infrared light (700–900 nm).[247] These two measures give a regional oxygen saturation reading for about 30% of the arterial and 70% of the venous components.[248] Near-infrared spectroscopy on the forehead calculates the $CrSO_2$ measurement.[249] The technology is based on the relative transparency of skin, bone, and connective tissue to near-infrared light, permitting estimation of cerebrovascular oxygenation and perfusion to a depth of 2–3 cm directly beneath the probe.[250,251]

Application of NIRS in GM-IVH prevention: Early detection of $CrSO_2$ provides real-time, continuous, and tissue-specific measures of oxygen saturation and tissue perfusion, enhancing current approaches to brain monitoring.[251] Cerebral regional oxygen saturation monitoring has provided evidence of a biphasic injury pattern, with an early rise in $CrSO_2$ due to hyperperfusion and a later drop due to tissue hypoxia.[250,252–254] Near-infrared spectroscopy may enable earlier recognition of the onset of cerebral hemodynamic changes, which is a crucial part of the pathogenesis of GM-IVH.[255] Real-time data can help clinicians know the right time to step in to prevent/diminish the likelihood of GM-IVH.[256] In one study, $CrSO_2$ trends in extremely preterm infants with GM-IVH during the first 72 hours of life were different from those of those without GM-IVH.[253] Another study showed that preterm infants with any grade of GM-IVH showed lower $CrSO_2$ for up to 4 weeks than non-IVH infants.[257]

Interventions guided by NIRS: Interventions that can be done based on NIRS, such as changing the ventilation parameters or implementing neuroprotective strategies, have promising results in preventing GM-IVH in preterm infants.[256] A recent report suggested that early detection of the fluctuations in $CrSO_2$ using a machine-learning model may help in the early initiation of therapeutic measures.[258] In normal infants, $CrSO_2$ values may vary depending on their gestational and chronological age and clinical condition.[259] Serial tracks may be more helpful than point measurements.[260] Near-infrared spectroscopy may be more useful with other monitoring systems for assessing cerebrovascular autoregulatory functions.[261,262] It should be viewed as an adjunct tool for monitoring.[250]

Challenges and Future Directions

Data interpretation and standardized monitoring protocols are the main challenges of implementing NIRS in neonatal care.[250] Standardized guidelines should be integrated into clinical practice. Further research is needed to assess the efficacy and cost-effectiveness of implementing NIRS-guided strategies to reduce GM-IVH.[263]

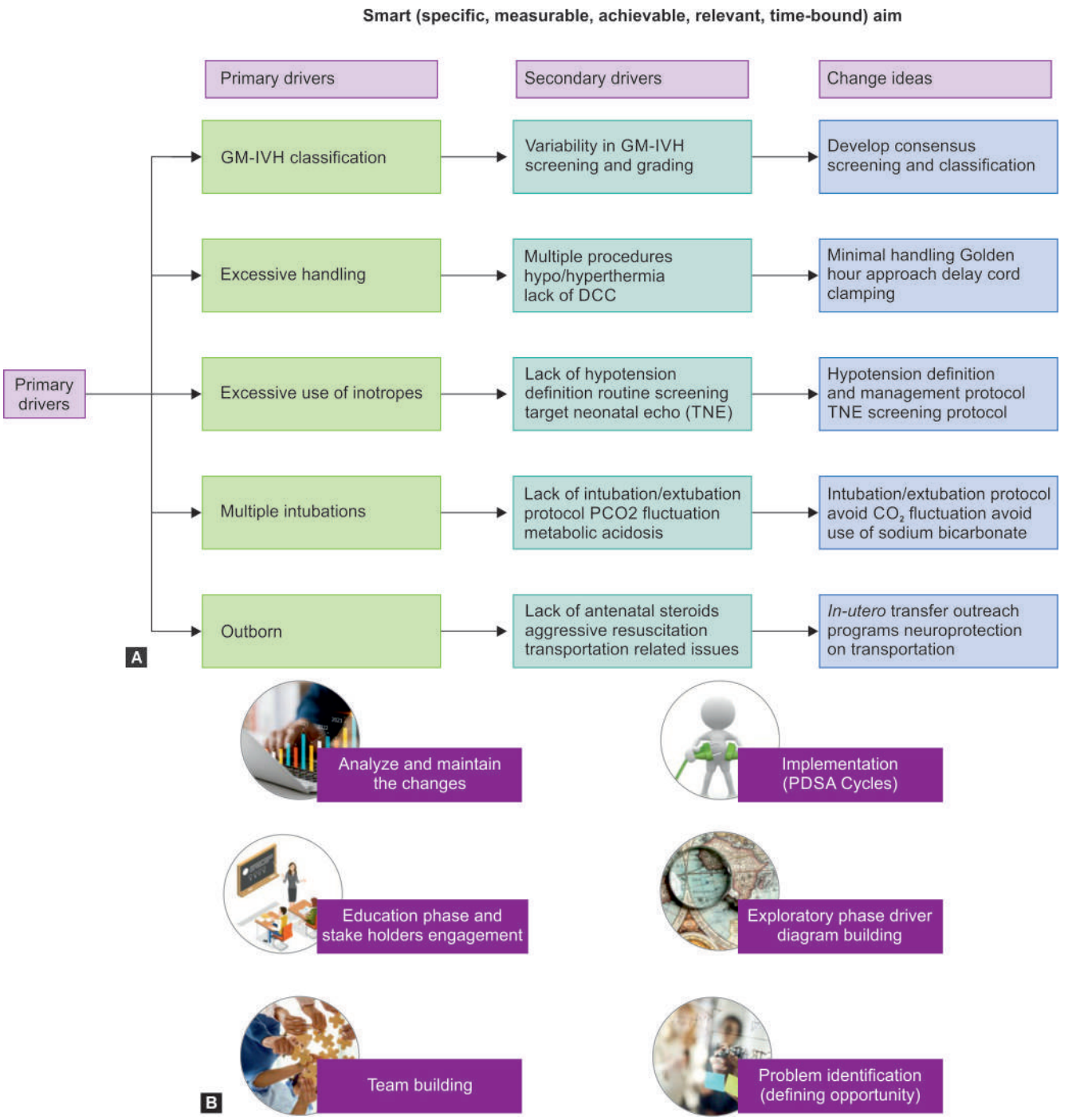
Technological advancement: Newer developments in NIRS technology, like broadband optical spectroscopy, have the potential to offer further information besides regional tissue oxygenation.[264,265] Such innovation could augment NIRS in preventing GM-IVH and other neonatal morbidities.[266] In conclusion, NIRS provides continuous information on cerebral oxygenation and blood flow and thus can direct targeted interventions to decrease the extent and prevent the occurrence of GM-IVH.[267] Considering the apparent safety of these non-invasive measurements, there is a high likelihood of widespread acceptance in routine, continuous measurements of cerebral hemodynamics.[250] Further research and clinical trials are needed to determine the full potential of NIRS in preventing GM-IVH and optimizing neonatal care.

Implementation Science

The bundle approach can effectively improve clinical outcomes in NICUs when researchers with evidence implement it into practice.[268] A dedicated leader is key to implementing a successful, potentially better practice. It is also vital to identify the areas for improvement within the unit, foster a sense of shared purpose, and collectively put it into action. When team members become aware of the issue and the QI initiative, they elaborate effectively to develop solutions and drive a positive drive to change. This effort is crucial for progress, leading to better outcomes: "The potential for positive change is immense and within our reach." Another important concept is optimal timing and sequence for implementing these bundles in NICUs, focusing on strategies of recommendation to improve the standardization of care bundles across multiple NICUs, with an eventual goal of consistency and reduction in variation. However, a high level of compliance is essential. Several ways have been described in the literature to improve the compliance of each care bundle. Once the area of improvement is determined and the primary target is set, a protocol will be developed based on evidence, followed by educational sessions for healthcare workers and providers. A systematic review of 47 studies conducted by Borgert et al.[269] showed that developing protocols, education materials, reminders, audits, and feedback are important strategies for implementing successful care bundles (Fig. 2).

Conclusion

The critical window period for brain injury is the first 72 hours of life, when 90% of premature infants are at risk of intraventricular hemorrhage. Most occur during the first 24 hours of life.[195] Based on assessed risk (Fig. 3), various interventions have been tried to decrease poor neurological outcomes during the pre-conceptional period (pre-implantation genetic diagnosis, folic acid supplementation), embryogenesis (avoidance of teratogenic drugs during critical stages of neural development), fetal period (treatment of chorioamnionitis, use of antenatal steroids and magnesium sulfate), delivery room interventions (deferred or delayed cord clamping, cesarean section, optimal thermal management techniques), neonatal (brain protection bundle of care), infantile period (early identification of high-risk infant, use of brain imaging, general movements assessments, neurodevelopmental screening such as Bayley's scales, ages and stages questionnaire, and initiation of early intervention) and beyond (disability limitation and supportive measures by screening for neurosensory impairments and correcting them). In this article, we discussed and reviewed the evidence of the major



Figs 2A and B: Strategies for successful implementation of care bundles. The need for platelet transfusions should be evaluated in infants with severe thrombocytopenia

neuroprotective bundles of care during the neonatal period. There is wide variation in perinatal bundles used for extremely premature neonates, differences in gestational ages and rates of follow-up, the developmental assessment tools used, and recommendations for best practices with good external validity. Pooling of data is recommended even with such variations for direct comparisons and benchmarking, and successful harmonization to allow the study of rarer complications. Interventions can potentially directly impact brain injury (hemodynamic and respiratory management) or indirect impact (light, noise, thermoregulation, handling, and vascular access). The success of getting good institutional results depends mainly on staff awareness of the risk factors and bundle elements, and compliance with these GM-IVH bundles. It is important to focus on sustaining the neuro-bundle to achieve a consistently improved quality of care. The potential for continuous improvement should inspire us all.[270]

Tables 2 to 4 summarize the information provided to standardize the interventions to reduce the incidence and severity of GM-IVH.

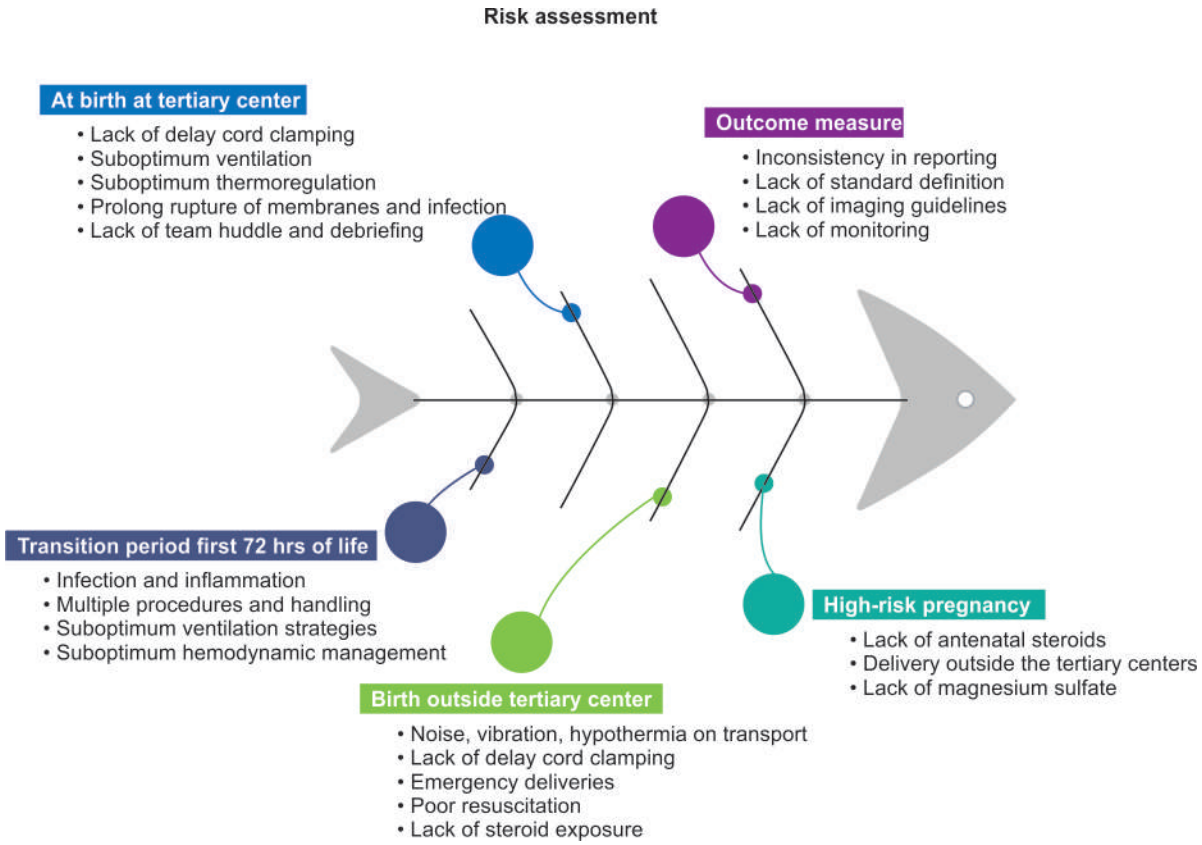


Fig. 3: Possible risk factors for GM-IVH based on postnatal age. Thrombocytopenia and coagulopathy are not included in this figure, as these factors are not specific to neonates and can increase the risk of intracranial hemorrhage in all age groups

Table 2: Strategies to improve standardization of GM-IVH care bundle

Antenatal care	• The *in-utero* transfer is always ideal.
	• Routinely administering antenatal corticosteroids 24 hours to 7 days before birth to all mothers expected to deliver a premature infant ≤ 34^{+6} and more than 22^{+6} weeks GA while those between 35 and 36^{+6} weeks GA in select clinical situations
	• Consider magnesium sulfate for all women experiencing imminent preterm delivery (≤ 33 + 6 weeks GA)
	• Outreach program
Perinatal care	• Golden hour
	• Delay cord clamping
	• Infants are managed on primary CPAP in the delivery room (except for infants born at <24 weeks' gestation)
Patient positing	• Maintain a neutral head position (head/neck in alignment with the body) when the infant turns and positions
	• Tilt the incubator to achieve 20–30º of upper body elevation during the first week of life
	• Avoid the baby's prone position during the first 72 hours of life
	• Avoid extreme 90º head turning
Nursing care	• Always question the necessity of care procedures
	• Experienced nurses must perform nursing care during the first week of life
	• Minimal handling with prioritizing the needs of the infant
	• Minimize routine care
	• Cluster care: Doing as much as tolerated by the infant (diaper changes, feeding, repositioning, etc.) at one time to allow the infant to have enough time for rest/sleep
	• Measure weight on admission and the fourth and seventh day of life
	• No length measurement for at least the first 72-hours
	• Nursing care and medical procedures should be combined and adapted to the infant's sleep-wake cycle
Care procedures	• Closed suction systems should be used on mechanically ventilated infants
	• The most experienced staff member must perform endotracheal intubation

(Contd...)

Table 2: *(Contd...)*

	• The blood samples from arterial lines with subsequent flashing should be drawn slowly • Avoid stress and pain • Evaluate stress and pain using pain scales • The first cranial ultrasound is better to be deferred at the end of the first week of life
Respiratory management	• Intubation should be selected based on clinical indicators according to the unit policy • Minimize extubation failure in the first 72 hours of life by ensuring that the baby meets extubation criteria outlined by the unit guidelines • Use volume target ventilation using an optimal lung strategy
Hemodynamic management	• Avoid inotropes if possible (allow for physiologic low blood pressure during first 72 hours from birth transition) • Have a clear guideline for when you use inotropes • Routine targeted neonatal echocardiography is not recommended in the first 72 hours unless intubated infant (ideally 24–48 hours), and there is concern for hemodynamically significant PDA
Thrombocytopenia and/or coagulopathy	• Need correction; target thresholds are still unclear—a balance between the lowest risk of bleeding and the minimum risk of inflammation. Further study is required

Table 3: Grade description for quality of evidence

Summary of recommendations with evidence

Grade	*Description of evidence*	*Certainty of evidence*
A	Strong - consists of studies from strong research	High
B	Moderate - consists of studies with a strong research design, but there are inconsistencies in results, generalizability, and/or risk/bias.	Moderate
C	Weak - studies show inconsistent results, and serious concerns about conclusions, generalizability, and/or risk/bias exist.	Low
D	A conclusion is either not possible or limited: Evidence is unavailable/or is of poor quality, and/or is contradictory.	Very low

Table 4: Summary of recommendations

Recommendation	*Description*	*Grade*	*Quality of evidence*
Antenatal steroid	Administration of antenatal corticosteroids to all the mother's expectant of delivery $\leq 34^{+6/7}$ weeks of gestation	B	II
Magnesium sulfate	Consider intrapartum magnesium sulfate for mothers who are at risk for imminent delivery of an infant $\leq 33^{+6}$ weeks GA in the next 24 hours	B	II
Delay cord clumping	Delayed umbilical cord clamping is recommended in preterm neonates	A	Ia
Mode of ventilation	Lung protective ventilation, which includes volume-targeted ventilation, should be the preferred mode of ventilation for all preterm infants during the first 72 hours of life	A	Ia
Head positioning	It should be neutral or in the midline during the initial 72 hours of life, along with the head of the bed raised to 20–30°	C	V
Nursing care	Nursing care during the first week of life must be performed by experienced nurses Minimal handling with prioritizing the needs of the infant Minimize routine care Measure weight on admission and the fourth and seventh day of life No length measurement for at least the first 72 hours	C	V
Care procedures	Closed suction systems should be used on mechanically ventilated infants Endotracheal intubation must be done by the most experienced staff member Drawing the blood samples from arterial lines with subsequent flashing should be drawn slowly	C	IIIa
Hemodynamic management	Avoid inotropes to treat hypotension unless a combination is associated with other signs, such as elevated lactate, prolonged capillary refill time, decreased urine output, or low cardiac output Care should avoid iatrogenic causes of hypotension, such as lung hyperinflation or dehydration Prophylactic indomethacin should be targeted to high-risk, extremely preterm infants, and the decision to treat should be based on combined risk factors	B	II

(Contd...)

Table 4: *(Contd...)*

Recommendation	*Description*	*Grade*	*Quality of evidence*
Umbilical cord milking	It is not recommended in very preterm infants <28 weeks due to increased risk for severe intraventricular hemorrhage	A	II
Platelet transfusion	It recommended a restrictive "low platelet" approach. Overall, there is limited effectiveness of platelet transfusions in reducing bleeding risk	A	II

***Quality of evidence classified as:**

- I: Systematic review with meta-analysis of homogenous randomized controlled trials (RCTs)
- II: Well-designed RCTs meta-analysis of non-homogenous RCTS
- III: Cohort or quasi-experimental trials
- IV: Descriptive
- V: Expert opinion or consensus

Additional lower-case letters are used: a: good quality, and b: lesser quality.

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ORIGINAL RESEARCH

Down Syndrome is the Leading Indication for Late-stage Termination of Pregnancy in Mongolia

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Abstract

Background: Prenatal sonograms frequently show congenital anomalies in fetuses. As expected, families receiving information about severe or multi-system anomalies experience ceaseless distress and may request for termination of the pregnancy. This study was designed to identify the most frequent indications for termination of pregnancy for fetal anomalies (TOPFAs). These data can help in early detection, which can then facilitate informed decisions either for safe terminations or for well-timed fetal procedures for rehabilitation. This information is also important for appropriate genetic testing and assessment of the risk of recurrence in later pregnancies.

Objective: To investigate the frequency and epidemiological profile of various fetal abnormalities that have evoked requests for late termination of pregnancy in Mongolia.

Materials and methods: This cross-sectional observational study was conducted in a cohort of 45,095 pregnancies. Of these, 156 were terminated because of fetal anomalies. Data pertaining to fetal/congenital anomalies were collected after informed consent from families and compared with 312 healthy controls to evaluate associated maternal risk factors.

Results: In this cohort, 34.5 in 10,000 pregnancies were terminated because of fetal anomalies during the study period 2017–2019. A total of 156 terminations were related to congenital anomalies. Down syndrome (DS) was the most frequent reason (25%). The other leading reasons were multiple congenital anomalies (16%), cleft lip/palate (10.9%), and anomalies of the central nervous system (9.6%) or the musculoskeletal system (9.6%). Maternal age >35 years, higher education, less spacing between successive pregnancies, and previous history of abortion(s) were associated with a higher likelihood of birth defects.

Conclusion: Down syndrome is the leading indication for late-stage TOPFAs in Mongolia. Multi-system congenital anomalies, clefts, and anomalies of the central nervous system and musculoskeletal system were other reasons that led to requests for termination of pregnancy.

Keywords: Birth defect, Chromosomal anomalies, Cleft lip, Cleft palate, Combined defect, Congenital anomalies, Down syndrome, European surveillance of congenital anomalies, International classification of diseases, Termination of pregnancy for fetal anomaly.

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Key Points

- Fetal/congenital anomalies include structural and/or functional defects in various organs or systems that are notable at birth.
- This cross-sectional observational study was conducted in Mongolia. In a cohort of 45,095 pregnancies, 156 were terminated because of fetal anomalies.
- Down syndrome (DS) was the leading reason (25%) for terminations. Multi-system anomalies (16%), cleft lip/palate (10.9%), and anomalies of the central nervous system (9.6%) or the musculoskeletal system (9.6%) were other frequent indications.
- Maternal age >35 years, higher education, less spacing between successive pregnancies, and history of abortion(s) were associated with a higher risk of birth defects. Identification of risk factors can help in designing appropriate interventions.

Introduction

Each year, there are about 133 million births all over the world. Of these, some 7.9 million show chromosomal and organ system defects. The prevalence of these defects typically ranges from 3 to 8% globally across races, ethnicities, and geographical regions.[1] An international survey across 193 countries showed a high prevalence of birth defects in Sudan at 82 per 10,000 live births, followed

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by Greece at 55, South Korea at 54, the United States (US) and China at 47, and France at 39.[2] The Global Burden of Disease study showed congenital anomalies caused 11% of all infant deaths[3] and considerable long-term impairment.[4] Unfortunately, consistent epidemiological data are not easily available in many regions. In this study, we focused on congenital disorders in Mongolia, both in terms of the spectrum and temporal changes, that have led to requests for termination of pregnancy.

Recent advancements in genetics and medical technologies have enabled early detection of anomalies *in utero* or soon after birth.[5] However, the etiopathogenesis of congenital anomalies remains uncertain in 40–60% of all cases. Single-gene disorders, chromosomal abnormalities, exposure to teratogens, and deficiencies in micronutrients may all be potentially important factors.[6] Maternal age, infections, concurrent chronic disease, *in utero* exposure to medical/recreational drugs, environmental contaminants, and radiation may also increase the risk and severity of anomalies.[7] There is a need for continued efforts to address these issues.

For fetal anomalies, timely identification, classification, and assessment for severity is important. The International Classification of Diseases (ICD) has emphasized severity as a key determinant in the identification of congenital problems.[8,9] Major congenital anomalies affect the infants' life expectancy, health status, or physical and social functioning; minor ones do not affect health or short-/long-term functions.[10–13] In contrast, anomalies classified as "minor" have minimal or no effects on morbidity or mortality.[10] Consistent prenatal monitoring and targeted care can effectively lower perinatal mortality.[11] The 63rd World Health Assembly passed a resolution to urge member countries to establish national programs for surveillance and prevention of congenital anomalies.[7]

In Mongolia, the incidence of anomalies has increased over the past two decades. During the period 1990–1999, 1.48 infants per 1,000 live births were noted to have congenital anomalies in the capital city region, Ulaanbaatar. This incidence increased to 2.15 in 2000–2004.[14] Improved quality and compliance with prenatal testing could be an important factor but other hitherto-unknown causes cannot be excluded. Improved perinatal care and increased fetal survival could also be one because many congenital abnormalities are associated with higher fetal mortality.[15] However, despite all advancements in prenatal care,[16] the medical establishment in Mongolia is not quite ready for *in utero* treatment of major anomalies. Hence, when conditions with the possibility of life-long disabilities are detected, termination of pregnancy is often seen as the most humane option. Similar to the laws in many other countries,[17,18] medical termination is permitted up to 22 weeks' gestation.[19]

Here, we have examined the indications and temporal trends of terminations of pregnancy for fetal anomalies (TOPFAs) in Mongolia. These data are needed for downstream studies to ascertain genetic and/or environmental risk factors/causes and socio-demographic associations. An improved understanding of the epidemiology of birth abnormalities can help design targeted preventive/therapeutic efforts.[20]

Materials and Methods

This cross-sectional observational study was performed between January 2017 and December 2019 in the Capital Hospital and the Amgalan Maternity Hospital, Ulaanbaatar, Mongolia. The ethics review committee approved the study prior to its initiation. Before enrolment for data collection, parental consent was obtained, strict confidentiality was maintained during data processing and the reports were created after removing all personal identifiers. Data were collected from mothers who were citizens of Mongolia and underwent TOPFA after 13 weeks of gestation.

Detailed obstetric and demographic data related to the current and previous pregnancy(-ies) were evaluated in detail. The frequency, pattern, and severity of anomalies of congenital malformations along with the period of termination were noted as outcome variables. All relevant variables were compared with a set of controls (pregnant women who delivered a healthy infant during the study duration). The total number of patients, the number included, the number identified to have congenital anomalies, and the number of healthy mother-baby dyads were evaluated for various risk factors.[21]

Prevalence of the birth defect(s) was estimated per 10,000 births. We used the Statistical Package for the Social Sciences (SPSS) software, version 25.0 for analysis. Socio-demographic information, risk variables, and the frequency of congenital malformations were summarized.[22] Qualitative data are reported as frequency and percentage, whereas quantitative data are presented as mean and standard deviation.[23] Student's *t*-tests and analysis of variance were used to analyze differences between groups.[24] Binary logistic regression was performed to evaluate risk.[25] Non-parametric methods were used as required.[26] Statistical significance was determined as $p < 0.05$ after due consideration.[27]

Results

Out of the 45,095 pregnant mothers seen during the study period, 156 underwent termination of pregnancy because of fetal anomalies. These were compared with 312 control mother-baby dyads to identify the risk factors associated with congenital anomalies. The recorded anomalies encompassed a range of conditions, including cardiovascular, musculoskeletal, chromosomal abnormalities, and neurological anomalies. Overall, congenital anomalies were seen in 0.35% of all patients.

The study included women aged 16–45 years. In the control group, the mean (± standard deviation) age was 27.9 ± 5 years, whereas the group with congenital anomalies was 31.8 ± 6 years. Table 1 shows the demographic characteristics of mothers who underwent TOPFA vs controls. Maternal age and education status were important; mothers who had an age >35 years and had received higher education were at greater risk of having fetuses

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Table 1: Maternal demographic profile

Maternal parameter	*Cases (n = 156) n (%)*	*Healthy volunteers (n = 312) n (%)*	*p-value*
Age (years)			
16–20	2 (1.3%)	12 (3.9%)	0.001
21–25	23 (14.7%)	89 (28.5%)	
26–30	51 (32.7%)	122 (39.1%)	
31–35	28 (16.7%)	63 (20.2%)	
36–40	38 (24.4%)	25 (8%)	
>40	14 (9%)	1 (0.3%)	
Education			
Illiterate	0	1 (0.3%)	0.03
Primary	0	1 (0.3%)	
Secondary	41 (26.3%)	106 (34%)	
Professional and technical	0	7 (2.2%)	
Higher	115 (73.7%)	197 (63.1%)	
Working status			
Unemployed	51 (32.7%)	129 (41.3%)	0.96
Employed	99 (63.5%)	149 (47.6%)	
Student	6 (3.8%)	34 (10.9%)	

Table 2: Maternal age-based termination of late pregnancy due to fetal anomalies

Maternal age	*Total births*	*Number of cases of late termination with fetal anomalies*	*Cases of late termination with fetal anomalies per 10,000 births*
<19	5,479	2	3.6
20–29	13,747	74	53.8
30–39	15,996	66	41.2
>40	9,873	16	16.2
Total	45,095	156	34.5

Table 3: Late terminations of pregnancy in the birth cohort by year

Year	*Total births*	*Number of cases of late termination with fetal anomalies*	*Cases of late termination with fetal anomalies per 10,000 births*
2017	20,156	72 (46.2%)	35.7
2018	20,186	68 (43.6%)	33.9
2019 (1st quarter)	4,753	16 (3.8%)	33.7
Total	45,095	156 (100%)	34.6

with birth defects, and consequently, underwent terminations of pregnancy more frequently than healthy controls. When considered individually, mothers with age 20–39 years had the highest number of termination of pregnancy (Table 2). The overall prevalence of birth defects leading to terminations in the defined period (2017–2019) was 34.5 per 10,000 live births. This was distributed as 35.7, 33.6, and 33.4 per 10,000 births in the years 2017, 2018, and 2019, respectively (Table 3).

Table 4 shows the prevalence of the various congenital malformations (Fig. 1). Chromosomal abnormalities were the most frequent (29.5%, 10.2 per 10,000 live births), followed by fetuses with multiple birth defects that were not otherwise specified (16%, 5.5 per 10,000 live births), cleft lip and palate (10.9%, 3.7 per 10,000 live births), and abnormalities of central nervous system and musculoskeletal system (each accounted for 15%, 9.6 per 10,000 live births). Some other less frequently seen malformations involved the urinary tract, cardiovascular system, gastrointestinal system, eye and ear abnormality, genital defects, and respiratory system in the descending order of prevalence. More details of this pattern of congenital abnormalities are provided in Table 5. In the subgroup with chromosomal abnormalities, (DS; 8.6 per 10,000 births) was the most frequent; the other frequently seen abnormalities were cleft lip and palate (3.7 per 10,000 live births). Multi-system anomalies included malformations, where migration defects in the central nervous system were the most frequent (1.6 per 10,000 births); similarly, phocomelia[28] and abdominal wall defects were also noted (1.8 per 10,000 births).

Table 6 shows the relationship between congenital abnormalities and other maternal factors. Pregnancy spacing of less than 2 years or a history of previous miscarriages were noted as important predictor of congenital anomalies. Sixty-seven percent of the anomalies in terminated pregnancies showed defects that might not have been immediately life-threatening; 33% of anomalies in terminated pregnancies were perceived as immediately life-threatening and the other 67% might not have resulted in life-incompatible anomalies. In the subgroup likely to have high mortality, most had combined abnormalities not otherwise specified (n = 21) followed by central nervous system abnormalities (Table 7). These anomalies are listed in Table 8. The occurrence of neonatal life-incompatible abnormalities showed no correlation with any of the maternal risk factors (Table 9).

Discussion

This large population-based study is the first of its kind to enlist the congenital anomalies leading to requests for TOPFA(s) in Mongolia. We also have presented information on maternal risk factors and neonatal outcomes associated with congenital anomalies in Mongolia. During the study period from 2017 to 2019, the prevalence of congenital anomalies leading to late termination of pregnancy in this region was 34.5/10,000 births. The numbers remained similar during the study years, but these data need to be followed closely. The incidence of lethal congenital malformations can vary over time and across regions.[29]

Timely detection of congenital anomalies was influenced not only by specific diagnoses/biological factors but also by socioeconomic status, maternal age/education, and spacing between successive pregnancies.[30] Even though this cohort did not show significant differences in the outcomes of infants who were treated at family, district, or private hospitals, the need for comprehensive and accessible healthcare services for expectant mothers can still not be disregarded. Timely diagnosis is important. Counseling about the availability of treatment modalities, the likelihood of success of intervention, and the predictors of subsequent morbidity and mortality can provide families with emotional support that all decisions were made only after considering all possible options.[31] The importance of this longitudinal panoptic approach cannot be over-emphasized.[32]

In our cohort, DS was the single most important diagnosis (25%) that led to TOPFAs. Down syndrome is characterized by the presence of a full trisomy or some extra genetic material from chromosome 21.[33] It is a well-recognized, serious multisystem condition with

Table 4: Systemic congenital anomalies leading to late termination of pregnancy

Organ system	*Number of cases (n = 156) (%)*	*Per 10,000 births*	*Mean maternal age at diagnosis*
Chromosomal anomalies	46 (29.5%)	10.2	32.6 ± 2.3
Down syndrome	39 (25%)	8.6	35.8 ± 5.8
Multi-system birth defects**	25 (16%)	5.5	30.1 ± 6.5
Cleft lip and palate	17 (10.9%)	3.7	30.8 ± 6.3
Central nervous system	15 (9.6%)	3.3	27.1 ± 6.7
Musculoskeletal system	15 (9.6%)	3.3	30.9 ± 7.1
Urinary tract	8 (5.1%)	1.7	30.6 ± 6.2
Cardiovascular system	6 (3.8%)	1.3	30 ± 2.7
Gastrointestinal tract	5 (3.2%)	1.1	28.7 ± 6.8
Eye and ear defects	2 (1.3%)	0.4	32 ± 4
Genital defects	1 (0.7%)	0.2	35
Respiratory system	1 (0.7%)	0.2	31
Other anomalies*	15 (9.6%)	3.3	30.6 ± 5.3

*Other anomalies include congenital diaphragmatic hernia (2), inguinal hernia (4), cystic hygroma (2), conjoined twins (2), and facial dysmorphism (5).
**Multi-system birth defects were not identified with the most frequent manifestations of any chromosomal abnormalities

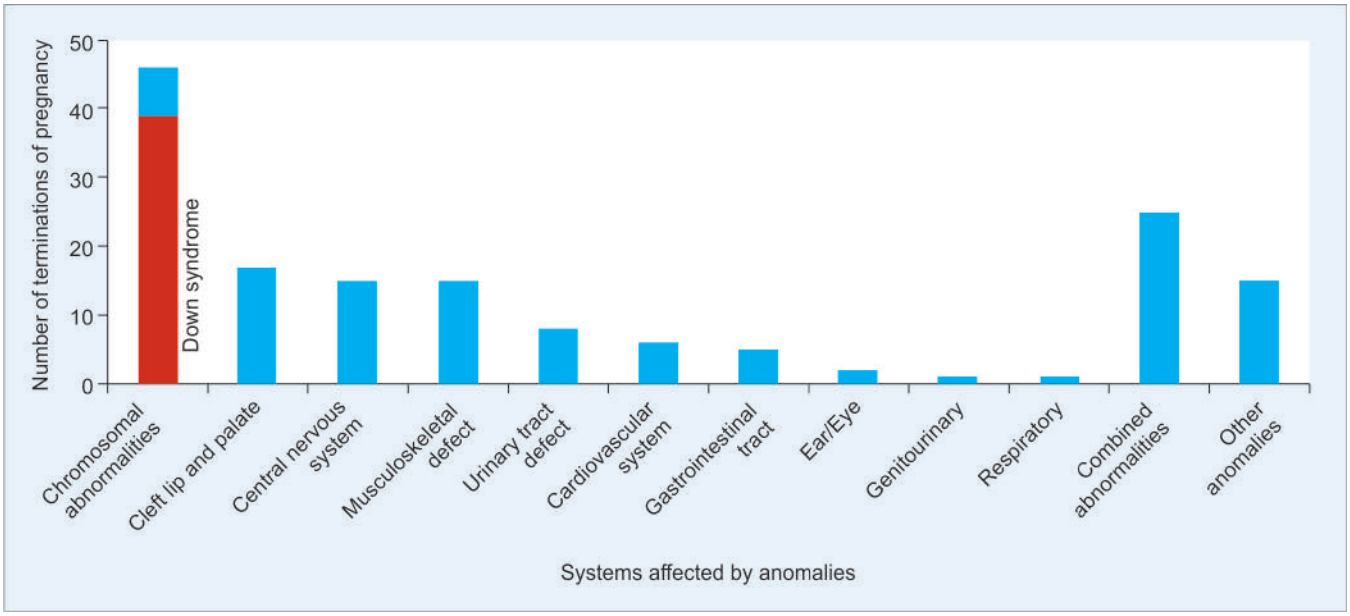

Fig. 1: Number of terminations of pregnancy performed for various systemic/genetic congenital anomalies during the study period. Down syndrome was the most frequently-noted chromosomal anomaly (depicted in red) in the first bar

life-long implications. The incidence is estimated to be somewhere between 1 in 1,000 and 1 in 1,200 live births worldwide,[34] indicating that about 3,000–5,000 infants are born with DS each year.[35] There can be minor temporal and geographical variability in geno-/phenotypic features but the condition has been constantly recorded in all races/ethnicities and economic levels.[36,37] Our understanding of the pathogenesis of DS remains limited. As is evident in our data and other studies, advanced maternal age is a known risk factor. The risk is known to increase with maternal age (1 in 1250 for a 25-year-old mother, 1 in 1,000 at age 31, 1 in 400 at age 35, and about 1 in 100 at age 40).[38,39] The average maternal age in our DS subgroup was 35.8 ± 5.8 years. In a retrospective review of the Asian population, Song et al.[40] highlighted that a maternal age of ≥34 years increased the risk of having an infant with DS. However, age is not the sole determinant; many infants with DS related to trisomy/structural abnormalities of chromosome 21 are born to women of age <35 years.[41]

Nearly 96% of all cases with DS have a supernumerary chromosome 21, resulting from a failure of these chromosomes to separate during gametogenesis.[42] All the cells in the body carry an extra chromosome 21, and consequently, an extra copy of >200 protein-coding genes affects homeostasis in cells, tissues, organs, and systems.[33] In 3–4% of cases, genetic alterations other than a canonical trisomy have been identified, including Robertsonian translocations, an isochromosome, or a ring chromosome.[43] The Robertsonian, or the translocation DS, is an unbalanced anomaly with an extra copy of the long arm of chromosome 21.[44,45] Isochromosomes contain long arms of the chromosome that mirror each other.[46] The ring chromosome 21 is seen less frequently; the ends of chromosome 21 join and form a ring.[44] Finally, 1–2% of infants with DS are mosaics, where some, not all, cells show a chromosome 21 trisomy.[47,48]

There is a need for detailed analysis of the chromosomal abnormalities in DS.[49] Phenotypical features such as the facial

Table 5: Congenital birth defects leading to termination

Anomaly	*Number of cases (n = 156)*	*Per 10,000 births*
Chromosomal (46; 29.4%)		
Down syndrome	39 (25%)	8.6
Edwards syndrome	4 (2.5%)	0.9
Other chromosomal syndromes	3 (1.9%)	0.7
Cleft lip and palate (17; 10.9%)		
Central nervous system (15; 9.6%)		
Brain malformations that are most likely to cause cerebral palsy (migration defect)	7 (4.4%)	1.6
Hydrocephalus	3 (1.9%)	0.7
Microcephaly	2 (1.3%)	0.4
Spinal deformities	2 (1.2%)	0.4
Cerebellar agenesis	1 (0.6%)	0.2
Musculoskeletal defect (15; 9.6%)		
Phocomelia	4 (2.6%)	0.9
Abdominal wall defect	4 (2.6%)	0.9
Foot deformities	3 (1.9%)	0.7
Skeletal dysplasia	2 (1.2%)	0.4
Brachydactyly	1 (0.6%)	0.2
Acromesomelic dysplasia	1 (0.6%)	0.2
Urinary tract defect (8; 5.1%)		
Bilateral polycystic kidney	3 (1.9%)	0.7
Megabladder	2 (1.2%)	0.4
Unilateral kidney cyst	2 (1.2%)	0.4
Unilateral kidney dysplasia	1 (0.6%)	0.2
Cardiovascular system (6; 3.8%)		
Ventricular septal defect	3 (1.9%)	0.7
Malposition of aorta	1 (0.6%)	0.2
Cor triatriatum	1 (0.6%)	0.2
Atrial septal defect	1 (0.6%)	0.2
Gastrointestinal tract (5; 3.2%)		
Tracheoesophageal fistula	2 (1.2%)	0.4
Hepatomegaly	2 (1.2%)	0.4
Esophageal and laryngeal perforation	1 (0.6%)	0.2
Ear/Eye (2; 1.3%)		
Microtia	1 (0.6%)	0.2
Microphthalmia	1 (0.6%)	0.2
Genital (1; 0.6%) Hypospadias	1 (0.6%)	0.2
Respiratory (1; 0.6%) Polycystic lung	1 (0.6%)	0.2
Combined abnormalities	25 (16%)	5.5
Other anomalies	15 (9.6%)	3.3

Table 6: Risk factors associated with congenital anomalies

Toxic	*Cases n (%) (n = 156)*	*Healthy volunteers n (%) n = 312*	*p-value*
Tobacco smoking	5 (3.2%)	5 (1.6%)	0.27
Alcohol	0	0	
Radiation exposure	15 (9.6%)	22 (7.05%)	0.35
Pregnancy spacing			
<2	70 (44.8%)	121 (38.8%)	0.01
3–5	42 (26.9%)	111 (35.6%)	
>5	44 (28.2%)	80 (25.6%)	
Previous miscarriages	63 (40.4%)	53 (17%)	0.001
Parity-primigravida	24 (15.4%)	62 (19.9%)	0.24
Consanguinity	25 (16%%)	65 (12.7%)	0.33
First antenatal care			0.88
<12 week	117 (75.1%)	233 (74.5%)	
13–20 week	20 (12.8%)	63 (20.1%)	
>21 week	9 (5.8%)	8 (2.7%)	
No antenatal care	6 (3.8%)	8 (2.7%)	
BMI			
<18.5	60 (38.5%)	100 (32.1%)	0.17
18.5–24.9	32 (20.5%)	95 (30.4%)	
25–29.9	33 (21.1%)	75 (24%)	
>30	31 (19.9%)	42 (13.5%)	
Type of Hospital			
Family hospital	19 (21.2%)	260 (83.6%)	0.21
District hospital	106 (77.3%)	51 (16.4%)	
Private hospital	2 (1.5%)	0	

Table 7: Anomalies compatible or incompatible with life

Organ system	*Compatible with life n (%)*	*Incompatible with life n (%)*	*Total*
Chromosomal anomalies	42 (91.2%)	4 (8.7%)	46
Combined abnormalities	4 (16%)	21 (84%)	25
Nervous system	1 (6.7%)	14 (93.3%)	15
Eye and ear defect	2 (100%)	0	2
Cardiovascular system	5 (83.3%)	1 (16.7%)	6
Respiratory defect	0	1 (100%)	1
Cleft lip/Palate	17 (100%)	0	17
Gastrointestinal tract	5 (100%)	0	5
Genital defect	1 (100%)	0	1
Urinary tract	5 (62.5%)	3 (37.5%)	8
Bone or musculoskeletal system defect	15 (100%)	0	15
Other anomalies	8 (53)	7 (47%)	15
Total	105 (67.3%)	51 (32.7%)	156

appearance, cardiac anomalies such as the endocardial cushion defect, neurodevelopmental delay, and the dermatoglyphic changes could all be rooted in the long arm of chromosome 21. The gene for the Cu/Zn-superoxide dismutase (SOD1) is located in 21q22.1,[50,51] the amyloid precursor protein (APP) in 21q11.2-21.05,[52] and six probes for single-copy sequences bind in a narrow, contiguous region: D21S46 in 21q11.2-21.05, D21S47 and SF57 in 21q22.1-22.3, and D21S39, D21S42, and D21S43 in 21q22.3.[53,54] The variability in the DS phenotype may result from the variability of gene expression of transcription factors that are encoded on chromosome 21, copy number polymorphisms, microRNA activities, RNA editing, and perhaps epigenetic modulation.[55] Understanding DS is a recognized, universal priority because this is not a universally lethal condition.[56] The United Nations General Assembly has marked 21st March as World Down Syndrome Day (A/RES/66/149)[35] to raise public awareness of DS. The DS International network

Table 8: Details of systemic anomalies perceived as compatible and incompatible with life

Organ/System	*Compatible with life*	*n (%)*	*Incompatible with life*	*n (%)*
Chromosomal abnormalities (46)	Down syndrome	39 (84.8%)	Edward syndrome	4 (8.7%)
	Other syndromes	3 (6.5%)		
Nervous system (15)	Meningocele	1 (6.7%)	Brain malformation	7 (46.7%)
			Hydrocephalus	3 (20%)
			Cerebellar agenesis	1 (6.7%)
			Microcephaly	2 (13.3%)
			Open cervico-dorsal meningomyelocele	1 (6.7%)
Ear defects (1)	Microtia	1 (100%)	–	
Eye defects (1)	Microphthalmia	1 (100%)	–	
Cardiovascular system (6)	Atrial septal defect	1 (16.7%)	Cor-triatriatum	1 (16.7%)
	Aorta malposition	1 (16.7%)	–	
	Ventricular septal defect	3 (50%)	–	
Cleft lip and palate (17)	Cleft lip—bilateral	12 (70.6%)	–	
	Unilateral cleft lip and palate	5 (29.4%)	–	
Gastrointestinal tract (5)	Tracheal-esophageal fistula	2 (40%)	–	
	Hepatomegaly	2 (40%)	–	
	Esophageal and laryngeal perforation	1 (20%)	–	
Genital tract (1)	Hypospadias	1 (100%)	–	
Urinary abnormality (8)	Megabladder	2 (25%)	Bilateral polycystic kidney	3 (37.5%)
	Unilateral kidney dysplasia	1 (12.5%)	–	
	Unilateral kidney cyst	2 (25%)	–	
Bone defect or musculoskeletal system defect (15)	Phocomelia	4 (26.7%)	–	
	Brachydactyly	2 (13.3%)	–	
	Skeletal dysplasia	2 (13.3%)	–	
	Foot deformities	3 (2.8%)	–	
	Abdominal wall defect	4 (26.7%)	–	
	Acromesomelic dysplasia	1 (6.7%)		
Respiratory system (1)	–	–	Polycystic lung disease	1 (100%)
Combined abnormalities (25)	–	4 (16%)	–	21 (84%)
Other anomalies	–	8 (53%)	–	7 (47%)

Table 9: Correlation of compatibility of fetal anomalies with maternal age mother's age

	Mother's age						
	Less than 30 years old		*31–40 years old*		*Older than 41 years old*		
Compatibility with life	*n*	%	*n*	%	*n*	%	*p-value*
Compatible with life	49	31.4%	45	28.8%	11	7%	0.626
Incompatible with life	27	17.3%	20	12.8%	4	2.6%	
Total	76	48.7%	65	41.7%	15	3.2%	

hosted the 13th World Down Syndrome Day Conference at the UN headquarters on 21–22 March 2024 in New York.[57]

The World Health Organization (WHO) records show that 17–42% of infant mortality all over the world can be attributed to congenital anomalies.[58] This is a major, distressing area that every obstetrician/perinatologist has to deal with in her/his everyday clinical practice.[32] In 11 European Surveillance of Congenital Anomalies (EUROCAT) countries,[59] the average infant mortality rate associated with congenital anomalies was 1.1 per 1,000 births.[60] Notably, countries where TOPFA is not permitted, such as Malta and Ireland, reported higher rates of infant mortality due to congenital anomalies, with rates of 3 and 2.1 per 1,000 births, respectively. The rate of stillbirths associated with congenital anomalies was reported as 0.6 per 1,000 births. The average number of TOPFAs, at 4.6 per 1,000 births, was nearly three times more than the combined rates of stillbirths and infant deaths. Termination of pregnancy for fetal anomalies have also been seen to impact the prevalence of post-neonatal survivors with non-lethal congenital anomalies.[61] Since deaths due to congenital anomalies tend to occur during infancy or in early childhood,[62] the burden in years of life lost is higher—congenital anomalies ranked 14th among all causes of death.[63] Moreover, it is more tedious to get the prevalence pertaining to low-middle-income countries which either do not have a national registry or the termination is illegal and hence, not

documented. A study conducted in Southeast Asia revealed that out of 640 women who underwent medical termination of pregnancy, 245 cases were attributed to congenital fetal malformations, accounting for 38.2% of the total cases.[64]

In addition to DS, early or advanced maternal age is also associated with an enhanced risk of other congenital anomalies.[65] The higher incidence of these anomalies among educated mothers could be attributed to better awareness of newer diagnostic modalities, but biological factors are likely important. Decreased spacing (less than 2 years) among the subsequent pregnancies with previous abortion were certain other contributory factors in the current study. Paternal consanguinity, maternal malnutrition, obesity, inadequate prenatal care, smoking, prior abortion, past congenital abnormality in their fetus, prematurity, and low birth weight are some factors studied.[6,21,66] Public education on risk factors, maternal health, and early prenatal care is crucial. A better understanding of these issues can help with timely and improved intervention, with better maternal and infant outcomes.[67]

In our cohort, we also recorded a high frequency of multi-system anomalies that could not be classified into known syndromes (16%), cleft palate and lip (10.9%), central nervous system (9.6%), and musculoskeletal defect (9.6%). Other common malformations were related to the urinary tract, cardiovascular, and gastrointestinal systems. Among the anomalies of the CNS, migration defects (4.5%) with a high likelihood of future cerebral palsy form a major cohort. Phocomelia and abdominal wall defects are among the most common anomalies in the musculoskeletal system leading to TOPFA. In a retrospective study from the African Gulf registry assessing the incidence and risk factors of congenital anomalies, the highest incidence was observed in CVS (35%), followed by multiple congenital anomalies (21%), chromosomal/genetic anomalies (15%), renal anomalies (12%), CNS anomalies (n = 20; 6%), facial anomalies (14.4%), and other anomalies affecting the GIT, respiratory system, urogenital system, and skeletal system (7%). Additionally, multivariable regression analysis revealed associations between various specific congenital anomalies and factors such as multiple pregnancies, parity ≥1, maternal body mass index (BMI), and demographic factors including the mother's age, ethnicity, and infant's gender.[68] In another cohort, the circulatory system was most frequently implicated, followed by the neurological and musculoskeletal systems.[12] Between 1990 and 2019 in China, there was an increasing trend in the age-standardized incidence rate for congenital anomalies, with an average annual percentage change (AAPC) of 0.26% (0.11–0.41%). By 2019, this rate reached 148.12 per 105 person-years (124.03–176.33). Most of these congenital anomalies were heart defects, which showed an AAPC of 0.12% (−0.08% to 0.32%). Conversely, the age-standardized mortality rate for congenital anomalies exhibited a decreasing trend, with an AAPC of −4.57% (−4.97% to −4.17%), reaching 4.62 per 105 person-years (3.88–5.57) in 2019. Most mortality cases were associated with congenital heart anomalies, which had an AAPC of −3.77% (−4.35% to −3.19%). Additionally, the age-standardized disability-adjusted life years (DALYs) rate for congenital anomalies also showed a decreasing trend, with an AAPC of −3.74% (−3.95% to −3.52%), reaching 480.95 per 105 person-years (407.69–570.04) in 2019.[30] With the variations across various sites, a cross-sectional study from Bangladesh also showed a higher incidence of CNS and musculoskeletal/gastrointestinal defects.[66]

In countries where termination of pregnancy is legal, there is some variation in the definitions of types of anomalies for which termination of pregnancy is permitted. For instance, in the United Kingdom (UK), the law allows termination if the anomaly poses a 'substantial risk' that the child would be "seriously handicapped."[31] Conversely, in the Republic of Ireland, 2 medical practitioners must agree that the baby will die during pregnancy, labor, or within 28 days after birth before permitting TOPFA.[69] In the United States, access to TOPFA varies by state due to differences in governments, healthcare providers, and medical insurance restrictions.[70] Six states entirely prohibit TOPFA, while an additional four requires mandatory counseling on available perinatal hospice services before it can be performed.[71]

In Mongolia, TOPFA during late pregnancy is permitted by law.[72] However, currently, there is no standardized registration and reporting system for these cases. There is awareness that infant mortality can be reduced through prevention, early diagnosis, and surgical care of birth defects. However, with these established laws, many fetuses who could have survived might have also got terminated; possible reasons could have been rooted in the lengthy process of evaluation, parental wishes, and financial difficulties. In the current study, 67.3% of fetuses were terminated despite the condition(s) being compatible with life, such as DS and cleft lip/palate. There is an urgent necessity for a comprehensive, legally-supported requirement for mandatory counseling for parents prior to any decision regarding termination. Such a system should provide parents with information about both antenatal and postnatal treatment options available along with financial support. This approach will likely reduce the termination in conditions that are potentially salvageable.

In a cross-sectional study involving over 3.3 million infants in the Czech Republic,[73] the combined incidence of major congenital heart defects during both prenatal and postnatal periods remained more or less the same over the three decades studied. Of these cases, 43% were born, 54.1% resulted in termination of pregnancy, and 2.9% ended in prenatal death. However, there were significant changes in the rates of detection and termination practices over time. Prenatal detection increased substantially from 6.2% in 1991 to 82.8% in 2021. Conversely, the rate of terminations decreased from 70% in 1991 to 43% in 2021. Most terminations were performed in the first trimester (73.3%) followed by a significant decline in the second (42.6%). Furthermore, there was also a decline in the postnatal prevalence of major CHDs from 0.21 to 0.14%. An individualized approach to healthcare can be helpful. Importantly, this indicates a degree of consensus about how appropriately trained health professionals, compassionate and person-centered care, good information and communication, and a thoughtful and integrated care pathway, can help make parents feel supported and cared for through what is an emotionally traumatic experience.[74]

We need standardized systems such as the ICD-9 or ICD-10 for categorizing birth defects.[8] These can enable data comparison across different geographical regions and over time. According to the Global Burden of Disease Study and WHO reviews, congenital anomalies may contribute to up to 17–42% of infant mortality.[61] Given the substantial variability in the incidence of congenital abnormalities across regions, efforts should be directed toward more precise targeting and intervention strategies.[75]

Conclusion

Chromosomal abnormalities such as DS and multiple congenital defects are the most common reasons for requests for TOPFA.

Congenital anomalies are noted more frequently in mothers with higher age, less pregnancy spacing, and a history of previous abortion. These congenital defects carry an emotional, financial, and social burden with a need for prolonged medical care and possible long-term disabilities; the effect is noticeable not only on a child's health and development, but also on families, healthcare systems, and the whole society.[76] Appropriate prenatal, intrapartum, or postnatal evaluation could be helpful in prevention, counseling, and management. Better understanding could help design studies aiming for genetic understanding, clinical amelioration, and even cure.

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ORIGINAL RESEARCH

Safety of Full Enteral Feedings Initiated Soon after Birth Instead of Parenteral Fluids in Clinically Stable 30–34 Weeks Gestation Premature Infants

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Abstract

Background: Many neonatal intensive care units use feeding protocols where infants born at 30–34 weeks' gestation are maintained exclusively on parenteral fluids for variable periods without enteral feedings, until there is confirmed hemodynamic stability without any doubt. In addition to the pain and discomfort, intravenous infusions are associated with an increased risk of hospital-acquired infections, which makes it an undesirable practice if not essential.

Objective: In this quality improvement (QI) effort, we tested the safety and efficacy of enteral feedings starting within the first 2 hours after birth in infants born at 30–34 weeks' gestation.

Materials and methods: Instead of intravenous fluids, we initiated fluid management in infants born at 30–34 weeks' gestation using oral/nasogastric milk feedings at 70–80 mL/kg/day divided every 3 hours, with 5 mL increments every 12–24 hours until 200 mL/kg/day was achieved. We compared the utilization of parenteral fluids, the incidence of infection, and growth before and after initiation of this new feeding policy.

Results: In our experience, these infants tolerated and utilized enteral feedings well with stable growth and biochemical parameters. They also tolerated daily volume increments in the enteral feedings. We did not find any hypoglycemic events as the first enteral feeding was administered within 2 hours after birth. The enterally fed group showed a similar safety profile with similar weight at discharge and weight Z-scores. We report that infants born as early as 30 weeks gestation can safely tolerate ab initio full enteral feedings.

Conclusion: Enteral feedings beginning within 2 hours after birth are a safe and efficacious strategy for fluid management in premature infants born at 30–34 weeks gestation. Routine use of parenteral fluids is not necessary in the initial management of these infants.

Keywords: Central venous lines, Early oral feeds, Hospital-acquired infections, Late premature infants, Newborn, Neonate, Nutrition, Parenteral fluids, Umbilical lines, Z-score.

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Keypoints

- In most neonatal intensive care units (NICUs) across the world, premature infants born at gestational ages higher than 34 weeks of gestation receive full enteral feedings. However, those born at 30–34 weeks' gestation typically receive intravenous fluids and if stable, some trophic feedings.
- In our NICU, we have treated clinically stable premature infants born at 30–34 gestation also with full enteral feedings starting soon after birth.
- We typically start at total fluid volumes of 70–80 mL/kg/day divided every 3 hours, with 5 mL increments every 12–24 hours until we achieve a total volume of 200 mL/kg/day. Mothers own milk (MOM) is always our first choice.
- Here, we report two periods where we used this full enteral feeding protocol. Both periods showed safety; the weight at discharge and the change in weight Z-scores were the same in both groups. We report that infants born as early as 30 weeks' gestation can safely tolerate full enteral feedings.

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Introduction

Many clinical practices in neonatal care are rooted in tradition and have not been critically examined for safety. One example is the initial "clinical stabilization" of premature infants where these infants first receive parenteral (intravenous) fluids during the first week and are started on enteral feedings only when they prove hemodynamic stability beyond doubt.[1–3] There is now some acceptance of enteral feedings ab initio in infants born at or beyond 34 weeks of gestation. However, there is continuing hesitation in enteral feeding in infants born at 30–34 weeks of gestation who might have mild-to-moderate respiratory distress; the fluid administration protocols

for these infants in many neonatal intensive care units (NICUs) are largely based on parenteral fluids. The use of excess fluids in the first week of life is also not uncommon. We have been concerned that in addition to the pain and discomfort, intravenous infusions are associated with an increased risk of hospital-acquired infections, which makes it an undesirable practice if not essential.[4–7] Some NICUs have started providing trophic feedings (≤20 mL/kg) to a subset of infants who are perceived as most stable.

In this study, we describe our experience with enteric administration of fluids starting on the first 2 hours after birth in infants born at 30–34 weeks of gestation.[8] Similar practices have also been tried elsewhere; they concluded that early and exclusive enteral nutrition increases the number of full enteral feeding days. This feeding practice also appears to improve fat-free mass accretion, increase length, and reduce hospitalization costs.[9] In 2024, a similar quality improvement (QI) effort was performed in India.[10] The clinicians there noted that early total enteral feeding in sick 27–32 weeks gestation preterm neonates led to early attainment of full feeds and birth weight, shorter hospital stay, and reduced incidence of bronchopulmonary dysplasia (BPD) as well as retinopathy of prematurity (ROP). They did not observe any increase in feeding intolerance, necrotizing enterocolitis (NEC), sepsis, or mortality.

In the end of 2017, we developed a feeding protocol to rationalize the use of parenteral fluids in infants born at 30–34 weeks gestation in the Neonatal Unit of the Clínica del Country. We started using oral/nasogastric milk feedings, not intravenous fluids at 70–80 mL/kg/day divided every 3 hours, with 5 mL increments every 12–24 hours until 200 mL/kg/day was achieved.[11–13] In stable infants with no respiratory distress, these feedings were administered orally. In patients with mild-moderate respiratory distress, the feedings were administered using an oro-/nasogastric tube. We did not administer parenteral fluids unless the patient could not tolerate the oral feeds or had another serious pathology requiring us to not start feedings. Intravenous lines were placed only if needed to administer medications. Whenever possible, mothers' own milk (MOM) was the fluid of choice; it was fortified per our protocols (>=100 mL/kg/day). We do not have access to a human milk (HM) bank or HM-derived HM fortifiers.

In this study, we compared the utilization of parenteral fluids, the incidence of infection, and growth before and after initiation of this new feeding policy. Both periods showed safety with similar weight at discharge and weight Z-scores. We report that infants born as early as at 30 weeks of gestation can safely tolerate ab initio full enteral feedings.

Materials and Methods

The present study reports the results of a quality-of-care effort; this is a cohort study before and after an intervention. This study was carried out using the EpicLatino data collection instrument in our unit that has authorization from the ethics committee with the exception of informed consent because it uses data from unidentified clinical records. Our goal was to compare the clinical outcomes prior to and after the adoption of these new feeding protocols.

We reviewed the medical records of all premature infants born at 30–34 weeks' gestation in two time periods, the first from 01/01/2010 to 12/31/2017 and the second, after satisfactory acceptance of the feeding protocols, from 01/01/2018 to 08/15/2022. Babies born in another institution prior to transfer were excluded.

Demographic characteristics, prenatal variables, such as intrauterine growth restriction (IUGR) and suspected chorioamnionitis, and postnatal variables such as the possibility of infection and respiratory distress of infants were noted.[14–16] The variables were presented in absolute or relative proportions, or in medians and interquartile ranges according to the nature of the variable. The number of cases with and without parenteral fluids, the incidence of infection defined by positive blood or cerebrospinal fluid cultures and the weight on admission and discharge. To assess the adequacy of nutrition, we compared changes in weight *Z*-scores between birth and discharge.[17]

Appropriate parametric and nonparametric statistical methods were used; the Chi-squared test with Pearson or Fisher's exact techniques for numerical, and the Wilcoxon rank tests were used for nonparametric variables, respectively.[18–20] For continuous variables that did not show a normal distribution, the Mann–Whiteney *U* or Kruskal–Wallis *H* tests were used.[21] A p-value < 0.05 was considered significant after due consideration.[22]

Table 1: Demographic and anthropometric characteristics of patients before and after policy implementation

	Before 2018		*After 2018*		
Gestational age	*n*	*%*	*n*	*%*	*p**
30	45	9	28	7	0.860
31	60	12	46	11	
32	85	16	62	15	
33	129	25	102	25	
34	199	38	164	41	
Total	518		402		
Birth weight					
500–749	1	0.2	0	0.0	0.891
750–999	10	1.9	7	0.8	
1000–1249	25	4.8	20	2.2	
1250–1499	70	13.5	46	5.0	
1500–2499	383	73.9	302	32.8	
2500–4499	29	5.6	27	2.9	
Total	518		402		
Sex					
Male	274	52.9	209	52.0	0.629
Suspected chorio[¥]	11	1.2	13	1.4	0.534
Destination					
Death	10	2.0	10	2.6	0.123
Home	487	94	382	95	
Congenital anomalies	29	5.6	25	6.2	0.436
NEC/perforation[β]	6	1.2	3	0.7	0,739

*Fisher's exact test; [¥]Suspected chorioamnionitis; [β]All 9 cases received parenteral fluids from birth since they were high-risk cases

Results

We identified 920 cases born at 30–34 weeks of gestation. The groups in the periods prior to and after the initiation of these QI efforts did not show any significant differences in demographic characteristics (Table 1). There was a signification reduction in the number of patients who received parenteral fluids from the first to the second study period from 425 (82%) before to 297 (26.2%) after implementation ($p < 0.0001$). The number of days of administration of intravenous fluids also decreased from a median (interquartile range) of 3 (4) to 0 (1); $p = 0.0001$. The weight at discharge and the

Table 2: Results

	Before 2018		*After 2018*		
Parenteral fluids	*n*	*%*	*n*	*%*	*p**
No	93	18.0	105	73.8	<0.00001
Yes	425	82.0	297	26.2	
Total	518		402		
Infection**					
No	509		400		0.129
Yes	9	2.1	2	0.5	
Total	518		402		
Days with parenteral fluids					
Median (IQR)	3 (4)		0 (1)		0.0001***
Change in Z-score between birth and discharge					
p50	−0.56		−0.55		0.910***
p25	−0.82		−0.83		
p75	−0.25		−0.23		
IQR	1.1		1.1		
Weight at discharge/ death					
500–749	1	0.2	0	0.0	0.5338***
750–999	3	0.6	2	0.5	
1000–1249	3	0.6	1	0.2	
1250–1499	2	0.4	6	1.5	
1500–2499	418	80.7	310	77.3	
2500–4499	90	17.4	82	20.4	
≥4500	1	0.2	0	0.0	
Unknown	0	0.0$	1	0.0	
Total	518		402		

IQR, Interquartile ranges; *Fisher's exact test; **Infection is defined as positive blood or spinal fluid culture; ***Kruskal–Wallis equality-of-populations rank test

change in weight Z-score were similar in both groups (Table 2). The number of infants with hospital-acquired infections (late-onset sepsis) decreased from 9 in the first period to 2 cases in the second period; this difference was statistically not different. There were no complications due to less use of parenteral fluids.

During the second period, the use of parenteral fluids decreased in all gestational ages, as follows: at 30 weeks from 93 to 68%, at 31 weeks from 95 to 54%, at 32 weeks from 89 to 31%, at 33 weeks from 82 to 22%, and at 34 weeks from 72 to 12%. There were no complications due to withholding parenteral fluids. No cases of persistent hypoglycemia requiring parenteral fluids were reported. There were fewer patients who developed sepsis; 9 cases in the 1st period vs 2 in the 2nd, but this difference was not statistically significant. The number of infants with NEC in the two groups was 6 (1.2%) and 3 (0.7%), respectively; all 9 had received parenteral fluids since birth as they were perceived to be at a higher risk. The three cases during the intervention period with NEC included two perforations in babies <1000 gms on postnatal day 6 with parenteral fluids since birth. The 3rd infant was one of a twin pair born with a birth weight of 1500 gms at 30 weeks of gestation. He had a high acuity of illness since birth and therefore, received a traditional parenteral fluid regimen. He developed NEC with perforation on postnatal day 7.

Discussion

This quality improvement study in our NICU showed that premature infants born at 30–34 weeks of gestation safely tolerated full enteral nutrition within the first two hours of birth. Even though the evidence for any benefits of enteral fasting is missing, many NICUs have treated IUGR "high risk" preterm infants NPO (nil per os) with intravenous fluids for a variable number of days; enteral feedings are then initiated and increased over a variable period.[23–25] Some units even suggest a 3–5 period of trophic feedings[26,27] but the effects have not been consistent.[28] In our NICU, we found that initiation with full-volume enteral feedings was safe. Since the adoption of this universal enteral feeding protocol, there has been a signification reduction in both the number of patients who received and the number of days of parenteral fluids, and there was no change in the weight at discharge.

The variability in feeding practices in premature infants continues in many NICUs even though advantages of early exposure to colostrum both in terms of immunological development and establishment of protective microflora, and in mother-infant bonding are now receiving attention.[29–32] In surveys in our EpicLatino group, many healthcare providers have expressed concerns about the possibility of complications, such as spontaneous intestinal perforation and/or NEC, even though epidemiological data now show decreased and delayed occurrence of intestinal complications such as spontaneous perforations/NEC. Most of the cases of NEC are anyways seen after the first week.[11,33–35] The antecedents/precipitants of NEC also might be changing.[35–37] There is a need for continuing medical education.

In our experience, preterm infants of 30–34 weeks of gestation tolerated and utilized enteral feedings well, as is apparent in their stable growth and biochemical parameters. They also tolerated daily volume increments in the enteral feedings. Notably, we did not find any hypoglycemic events as the first enteral feeding was administered 2 hours after birth. There were fewer systemic infections in the absence of intravenous lines, which is plausible and can likely be proven in a cohort of adequate size. In terms of the adequacy of nutrition, the two groups showed no difference in the weights at discharge. Consistent with these data, the *Z*-scores computed at birth and discharge were also not different. These figures suggest that the protocol-based advancement of enteral feedings is adequate for good growth in this population.

Conclusion

This QI study confirms that routine use of parenteral fluids is not necessary for initial management of preterm infants born at 30–34 weeks' gestation. Most of these infants can be managed with oral feeding started soon after birth. The value of oral/enteral nutrition is well-established; the composition of enteral feedings over that of intravenous fluids does not require major explanations. Early establishment of full enteral nutrition can shorten the length of hospital stay.[38–40] These effects have been correlated with improved weight gain, linear growth, brain growth,[41] neuromotor integrity, and even optimized neurodevelopment.[42] A larger sample size may confirm the differences in adverse events such as hospital-acquired infections.

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REVIEW ARTICLE

Camel Milk as a Source of Nutrients and Immunogens for Infants

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Abstract

Camel milk stands as a vital resource for infants in arid and semi-arid regions. Despite representing a modest 0.36% of global milk production, its nutritional composition is remarkable. With 3.4% protein, 4.4% lactose, and 3.5% fat, it offers a unique blend of nutrients that is comprised of higher levels of essential vitamins and minerals compared with cow's milk. Notably, its vitamin C content surpasses that of cow's milk by a significant margin. This nutritional powerhouse is particularly beneficial for individuals allergic to cow's milk, as it lacks β-lactoglobulin. Beyond its nutritional profile, camel milk contains nanobodies that stimulate immune responses, unsaturated fatty acids for heart health, and insulin-like proteins that are stomach-friendly. Moreover, its probiotic bacteria aid in reducing cholesterol absorption and possess antibacterial properties, further enhancing its health benefits. In essence, camel milk transcends its role as mere sustenance, emerging as a potent superfood with the potential to address various health complications.

Keywords: Arid, Atopy, Camel milk, Composition, Food security, Health, Nanobodies, Semi-arid, Superfood, Vitamin C.

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Keypoints

- Camel milk, despite its easy availability and myriad benefits as a nutrient for young infants, remains underutilized and deserves a close examination.
- Camel milk boasts a rich nutritional profile, with higher levels of essential nutrients compared with cow's milk, including vitamins C, B3, manganese, iron, copper, and zinc. It might be particularly beneficial for atopic infants who react to bovine milk as it lacks β-lactoglobulin.
- It contains lactoferrin and lactoperoxidase, which exhibit antibacterial, antiviral, and anti-inflammatory properties, potentially enhancing overall immune function and combating infection.
- Camel milk's diverse array of nutrients, immune-boosting properties, heart-healthy fats, and antibacterial components position it as a promising superfood with potential benefits for various health complications.

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Introduction

Camels are the fifth largest contributors to the global dairy industry after cows, buffaloes, goats, and sheep. Annually, these mammals yield around 2.91 million tons of milk, comprising nearly 0.36% of the world's total milk output.[1–3] There is some variability related to age, geographic location, pregnancy, nutrition, seasonal fluctuations, health status, and genetics,[4,5] but over an 8–18-month lactation period, camels can produce between 1,000 and 12,000 liters of milk. Camels can produce 6–12 liters of milk per day in a controlled environment, but when exposed to the hot, dry conditions in the peri-equatorial/tropical belt, the daily yield is typically around 2 liters.[4]

There are currently two major species of camels (Fig. 1), with a third endangered group with less than 1000 surviving animals.[3] The one-humped dromedary, which makes up 94% of the world's camel population, can produce up to 15–20 liters of milk. In this species, the Pakistani breed has the highest milk output. The two-humped Bactrian make up most of the other 6%; these animals produce less, only 0.5–1 liter daily. However, the two-humped animals from Kazakhstan can produce up to 6–7 liters/day to surpass local cows and one-humped camels.[1] Overall, there is a pattern of milk production across species; the output peaks around 4 months after delivery and then declines by about 50% in the 16th month.[1]

Camel milk is typically comprised of 89–91% water and has a lower viscosity than cow's milk; it serves as a crucial water source for camel calves in arid regions.[6] There are some similarities in composition with human milk but it differs considerably from the milk of other ruminants.[7] It has a creamy white appearance with a unique flavor profile combining sweetness with a subtle hint of spice. There may be a touch of salt, influenced by the desert plants consumed by camels. The pH of fresh camel milk typically ranges between 6.5 and 6.7, which is similar to that of sheep milk but is lower than cow' milk. These properties are likely influenced

Figs 1A and B: Camel species: (A) One-humped dromedary; (B) Two-humped Bactrian camels. A third species, the wild Bactrian camels (not shown) are close to extinction. The word "dromedary" is derived from the Greek word *dromas*, meaning runner. These camels originated in Arabia/Somalia. The Bactrian camels are named after the Bactria civilization in present-day Afghanistan/Iran

Table 1: Approximate chemical composition (gm%) of camel milk compared with other species[12]

	Water	*Protein*	*Fat*	*Ash*	*Lactose*
Camel	89–91	3–3.9	2.9–5.4	0.6–1	3.5–5.8
Cow	85–87	3.2–3.8	3.7–4.4	0.7–0.8	4.8–4.9
Buffalo	82–84	3.3–3.6	7–11.5	0.8–0.9	4.5–5
Sheep	79–82	5.6–6.7	6.9–8.6	0.9–1	4.3–4.8
Goat	87–88	2.9–3.7	4–4.5	0.8–0.9	3.6–4.2
Human	88–89	1.1–1.3	3.3–4.7	0.2–0.3	6.8–7

by higher levels of vitamin C and organic acids[8] and can facilitate iron absorption.[4,9] During storage, the pH drops with increased lactic acid content from 0.03% at 2 hours to 0.14% after 6 hours.[4] It has a lower buffering capacity and freezing point compared with cow's milk.[4]

On average, camel milk typically contains about 3.4 grams/dL (gm%) protein, 3.5 gm% fat, 4.4 gm% lactose, 0.79 gm% ash, and 7.9 gm% total solids suspended in water.[7] Notably, most amino acids, except lysine, glycine, threonine, and valine, are seen in higher concentrations in camel milk than in cattle.[7,10] The fat portion is typically comprised of 65–70% saturated and 30–35% unsaturated fatty acids (FAs). Compared with cows and other ruminants, camel milk boasts a more favorable ratio of unsaturated to saturated FAs.[10] There are more long-chain FAs, linoleic acid, and unsaturated FAs, which are important for nutrition and health, whereas the short-chain FA content is lower.[5,11] The composition of camel milk compared with other mammals is given in Table 1.[12]

Camel colostrum contains more proteins, peptides, vitamins, and minerals, and less lactose and fat compared with other animals.[8] It is produced for 5–7 days after birth and then transitions to mature milk.[8]

Composition of Camel Milk

Protein

Camel milk has a higher proportion of whey protein than cow's milk. The whey-to-casein ratio is lower than that in mare, monkey, and human milk but higher than in cattle, buffalo, sheep, and goat milk.[13] The casein content is similar to cow's milk. One exception is in milk from the 1-humped dromedary camel milk, which contains 1.6–2.67% of total milk protein. Beta-casein contributes 65%, alpha1-casein about 21%, and kappa-casein 3.47% to the total casein content.[14,15] The higher proportion of beta-casein in camel's milk is particularly advantageous for human health, as beta-casein is more easily digested and may be less allergenic due to its greater susceptibility to peptic hydrolysis in the gut.[16] The amino acid composition of camel vs human milk is summarized in Table 2.

Table 2: Comparison of free amino acid content in human milk vs camel milk (mg/mL; means)

Amino acid	*Human milk*	*Camel milk*
Alanine	4.2	3.3
Arginine	3.9	5.1
Aspartic	6.8	7.2
Cystine	1.2	1.5
Glutamic	16.6	21.1
Glycine	1.5	1.2
Histidine	2.6	2.9
Isoleucine	4.0	4.9
Leucine	8.9	9
Lysine	11.1	6.6
Methionine	1.3	2.6
Phenylalanine	3.2	3.7
Proline	10.2	13
Serine	3.2	3.0
Threonine	8.6	5.3
Tyrosine	2.6	3.0
Tryptophan	0.7	1.8
Valine	9.4	4.8

In camel milk, whey proteins comprise approximately 20–25% of total milk protein. Alpha-lactalbumin is the predominant whey protein, accounting for nearly 40% of total whey protein content; this contributes to its superior digestibility and enhanced

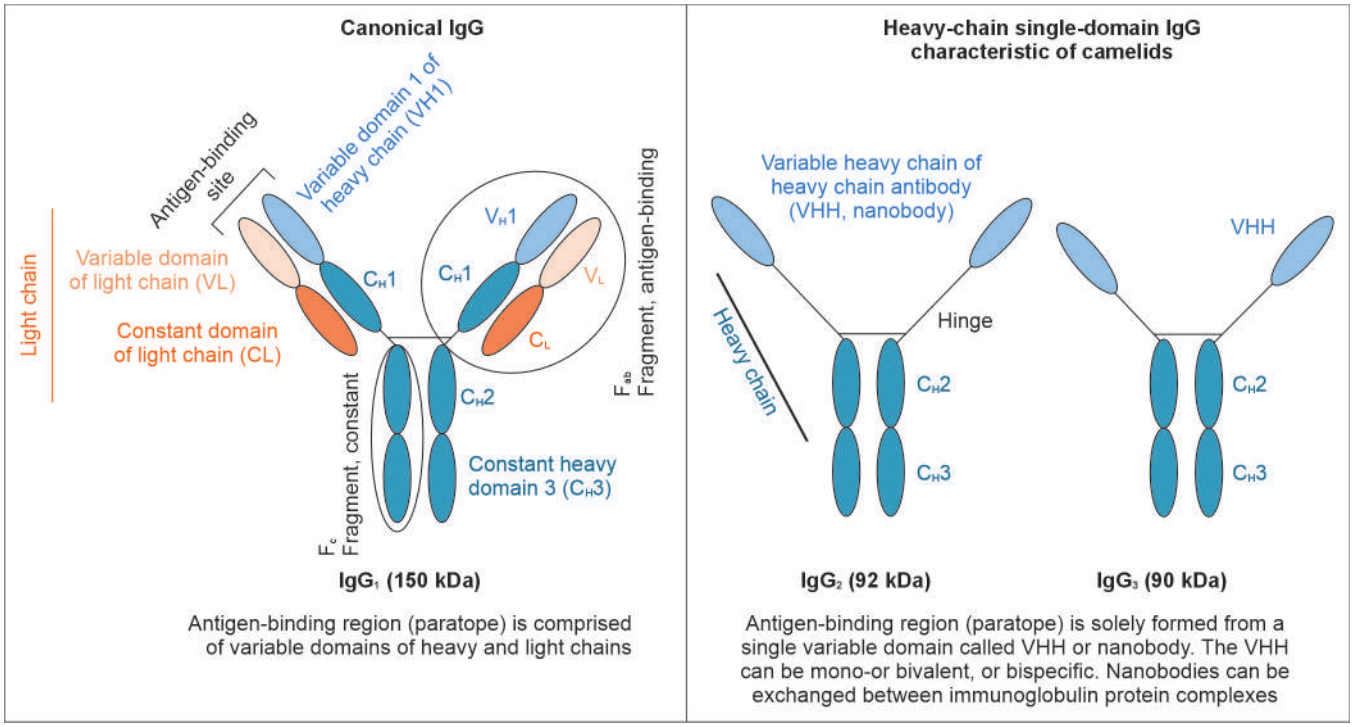


Fig. 2: Schematic showing the structure of IgG (IgG1, IgG2, and IgG3) antibodies in camel milk. Please note the absence of light chains in the Fab fractions of IgG2 and IgG3

antioxidant properties compared with bovine alpha-lactalbumin.[17] The primary sequences of camel and bovine alpha–lactalbumin show a high degree of congruence – the percentage sequence identity and similarity are 69% and 83% respectively, due to 39 different residues between both proteins.[18] However, the core of camel milk alpha–lactalbumin is more hydrophobic than that of the bovine protein.[19,20] Both proteins have the same number of cysteine at the same positions and the same number of disulfide bonds (Cys^{6}/Cys^{120}, Cys^{28}/Cys^{111}, Cys^{61}/Cys^{77}, and Cys^{73}/Cys^{91}).[18] Previous studies have shown that 123 amino acid residues in camel milk alpha-lactalbumin form a compact globular structure stabilized by four disulfide bounds, and that it exhibits a high affinity to calcium and other metal ions.[18] Furthermore, it is richer in essential amino acids and is more digestible than the corresponding bovine protein.[21] These characteristics make camel's milk a favorable choice for infant foods.[22]

Camel milk contains large amounts of immunoglobulins, insulin-like protein, and protective enzymes, such as lactoferrin, lactoperoxidase, and lysozyme.[1] Immunoglobulins, particularly IgG, are crucial components, with three main subgroups: IgG_1, IgG_2, and IgG_3. Notably, IgG_2 and IgG_3 lack light chains[23] (Fig. 2). Lactoferrin and IgG of camel's milk can inhibit the hepatitis C and B viruses and prevent their replication in cells.[24,25] The insulin-like protein could possibly help reverse maternal type 1 and 2 diabetes mellitus and gestational diabetes. Raw camel milk has immune-modulatory effects on the pancreas beta-cells, increases insulin secretion, and reduces insulin resistance in patients with type 1 diabetes.[26] It contains small-size immunoglobulins which strengthen the immune system. Hence, camel milk can serve as a unique superfood in many health issues of humans raising the utility benefits.[4]

Camel milk contains large-sized micelles (Fig. 3), which typically measure about 260–300 nm in diameter (compared with 100–140 nm

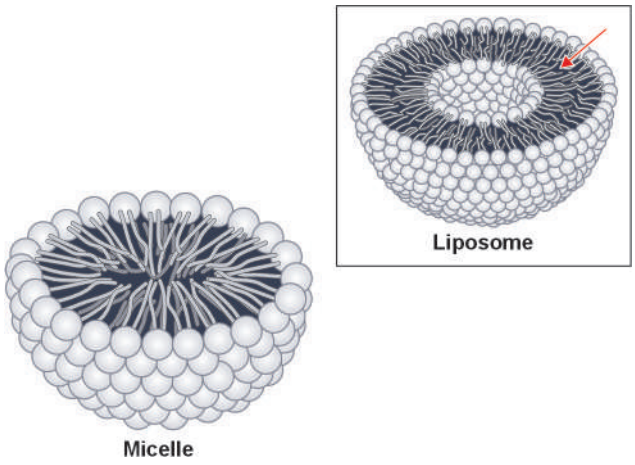
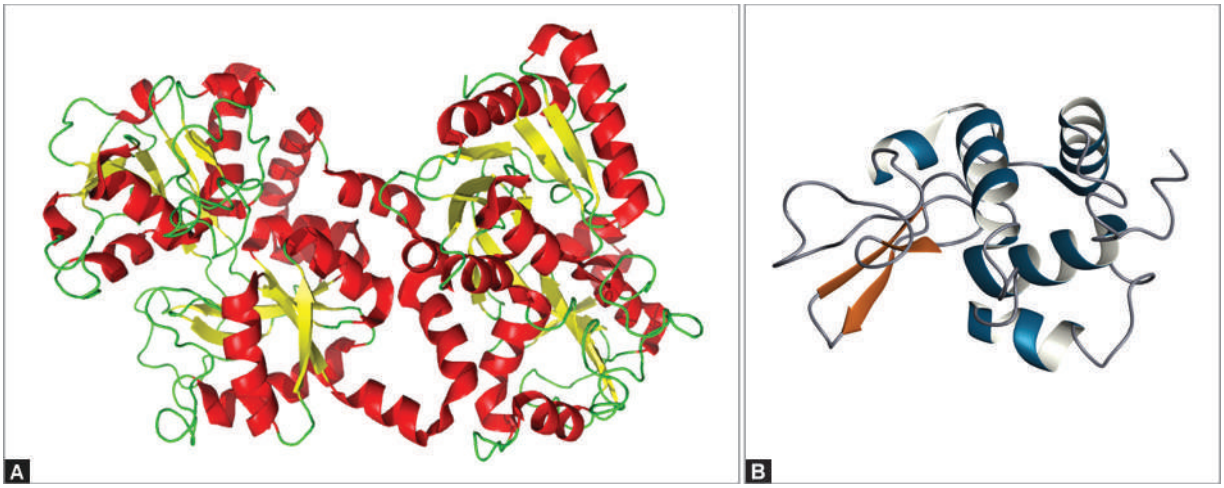


Fig. 3: Micelles in camel milk. A micelle is a particle of colloidal dimensions that exists in equilibrium with the molecules or ions in the solvent. The shape/size varies with surfactant concentration and physical conditions. These differ from liposomes (inset), which are small artificial vesicles that are covered in at least one lipid bilayer (arrow)

in cows),[6,27] and do not react with acid.[28] These micelles are rich in immunoglobulins (Ig), particularly IgG.[29] Overall, camel milk contains higher Ig concentrations than human milk. Interestingly, camelid (the biological family Camelidae, which includes dromedary camels, Bactrian camels, wild Bactrian camels, llamas, alpacas, vicuñas, and guanacos)[30] IgG molecules are small, only about 1/10th the size of human antibodies, and are therefore, easily absorbed across the intestinal barrier into the bloodstream.[31,32] These antibodies exhibit potent antiviral, antibacterial, and immunological effects.[33] Notably, the IgG_2 and IgG_3 subclasses of camelid antibodies lack light chains



Figs 4A and B: Camel milk contains important concentrations of (A) lactoferrin; and (B) lysozyme, which play key roles in the innate immune system

and show both higher efficiency (potency) and mechanical efficacy in binding antigens.[34] However, small variations in effectiveness can be seen with specific types of antigens.[35]

Camel milk also contains IgM, IgA, and IgD immunoglobulins.[1] These immunoglobulins can help prevent autoimmune diseases during pregnancy and promote immune system enhancement and regeneration. Camel milk antibodies are potent inhibitors of hepatitis virurses, particularly hepatitis C; the foot and mouth virus; Rift Valley fever virus; and the Rinderpest virus.[36] Immunoglobulins G in camel milk show important neutralizing activity against tetanus toxin by entering the enzymatic structures and neutralizing viral enzymes.[28] Fermented camel's milk contains bioactive peptides that may contribute to lowering cholesterol levels.[16] Additionally, camel's milk naturally contains orotic acid, which is known for its cholesterol-lowering effects in humans.[16]

Camel milk contains high concentrations of lactoferrin[37] (Fig. 4A), a key carrier of iron molecules that also plays an important role in the innate immune system.[38] It shows important antibacterial, antiviral, antiparasitic, catalytic, anticancer, and antiallergic functions; the concentrations are higher than that in cow's milk; 220 mg/L vs 110 mg/L.[39] Camel milk also contains more lysozyme (Fig. 4B), 288 mg vs 13 mg% in bovine milk.[40] Higher concentrations of lactoperoxidase, IgG, and vitamin C (than in cow's milk) further impart stronger antibacterial and antiviral properties.[41]

Traditionally, camel's milk has been used for the treatment of diseases like tuberculosis, asthma, dropsy, and jaundice owing to its content of natural bioactive components.[42] A study focused on knowledge, attitude, and practices in the UAE showed that 60% of the population had sampled camel milk but only 25.1% were consistent consumers. Besides fresh milk, yoghurt, and flavored milk were popular products. Consumers favored camel milk for its nutritional (66.4%) and medicinal (39.3%) properties. Many (58.4%) viewed unpasteurized camel milk as fresher (87.2%), immune-boosting (41.6%), and nutrient-rich (39.2%). Addressing misconceptions, especially regarding unpasteurized milk, is crucial for public health, necessitating national regulations.[43]

Unlike bovine, but similar to human milk, camel milk lacks beta-lactoglobulin.[44] The levels of beta-casein are also lower. These proteins, particularly beta-lactoglobulin, are important in the pathogenesis of atopy. Beta-lactoglobulin is a known allergen with exactly-mapped antigenic regions; its immunoreactivity has been associated with epitopes in (a) amino acid fragments 41–60, 102–124, and 149–162, which are recognized by 92, 97, and 89% of sera, respectively; (b) fragments 1–8 and 25–40, recognized by 58 and 72% of the population; and (c) peptides 9–14, 84–91, and 92–100, which are detected by more than 40% of sera.[45] Additionally, camel milk contains an acidic protein with protease inhibitor activity, and its lysozyme content is higher compared with cow, buffalo, sheep, and goat milk.[1,46]

Camel's milk, whether raw or in fermented dairy products, serves as a source of probiotic strains. Various species including *Lactobacillus*, *Bifidobacterium*, *Enterococcus*, and *Streptococcus* have been isolated from camel's milk.[47,48] Also, the angiotensin-converting enzyme (ACE) inhibitory activities demonstrated after enzymatic hydrolysis of milk proteins with alcalase and after fermentation of milk with *Lactobacillus* spp. showed potent antihypertensive properties.[49]

Fat

Camel milk typically contains 2.3–6.4 grams of fat per dL. The fat content and hence, energy content, is higher in milk from the two-humped Bactrian camels in cold desert environments than in the one-humped dromedary camels.[50] Camel milk contains small fat globules, which aid digestion and reduces the creaming properties.[51] Compared with cow's milk, there are fewer short-chain (1.2% vs 9% in cow's milk) but more long-chain FAs.[52] There are more unsaturated FAs, which explains the smooth appearance related to better homogenization.[1] These unsaturated FAs can lower serum lipids and promote cardiovascular health;[1] the atherogenicity index [content ratio of saturated/unsaturated FAs: (C12 + 4 (C14) + C16):(sum of the unsaturated FAs)] is better (2.7 in camel milk vs 3.3–3.5 of cow's milk).[53,54]

Camel milk contains high levels of conjugated linoleic acid, which makes it desirable in diets (1.2, 0.4, and 0.6 grams/100 grams of fat in camel, human and cow's milk respectively).[55] The metabolism is summarized in Figure 5. Omega-3 FAs in camel milk can promote growth, development, and possess anti-inflammatory properties.[10] Alpha-linolenic acid is converted into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA; Fig. 6), leading to a favorable lipid profile.[56]

The total cholesterol content in camel milk is higher than cow's milk (31.3 mg/100 mL vs 12–17 mg/100 mL).[53] The total phospholipid

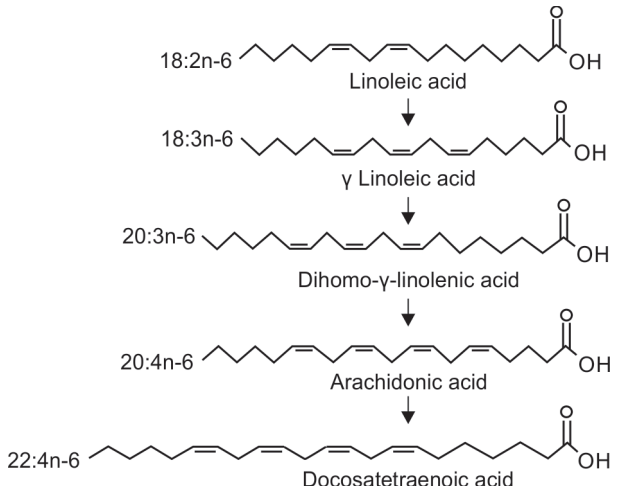


Fig. 5: Metabolism of linoleic acid

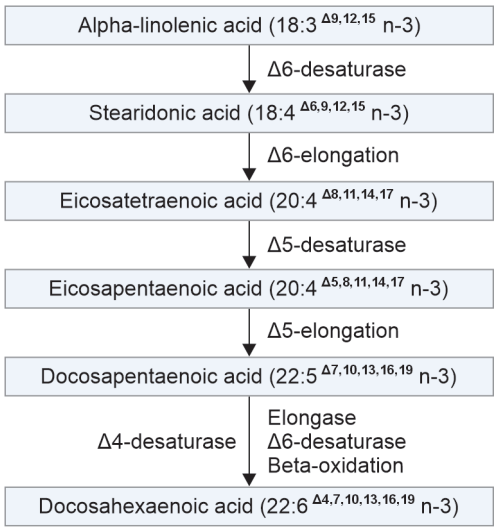


Fig. 6: Conversion of alpha-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid gives a favorable lipid profile

levels are also higher (0.5 mm vs 0.3 mm), with high concentrations of sphingomyelin (117.5 µg/mL) and plasmalogens (24 µg/mL).[57]

Minerals

Camel milk is rich in essential minerals such as calcium, iron, magnesium, copper, manganese, sodium, phosphorus, zinc, and potassium. Compared with cow's milk, iron, copper, zinc, and manganese concentrations are higher.[28] The iron levels, better than in cow's and goat milk, make it a desirable infant food as it can support infant growth and prevent anemia.[28,29,58] The calcium-to-phosphorus ratio in camel milk is 1.5:1, again better than other mammals (cow's milk 1.29:1); human milk scores better here at 2.1:1. These levels can help maintain serum calcium levels and prevent hyperphosphatemia.[28,59] The calcium concentrations in camel milk range between 30 and 257 mg/100 mL, phosphorus between 34 and 100 mg/100 mL, sodium 220 and 990 mg/L, and potassium at 520–1800 mg/L.[58] Copper content varies widely between 30 and 800 µg/100 mL, while zinc ranges from 0.06 to 0.50 mg/100 mL.[60]

Vitamins

Camel milk is rich in vitamins B_1, B_2, B_{12}, and contains significant levels of carnitine.[61] Niacin content in camel milk ranges from 11.6 to 520 µg/100 mL, about 5 times higher than in cow's milk.[58] However, the levels of B_2 (riboflavin) are only ¼th, B_9 (folic acid) only 1/13th, and B_5 (pantothenic acid) ¼th compared with cow's milk.[61]

Vitamin C levels are considerably higher than in other mammals (camel milk 52 mg/L vs 27, 22, 29, 16, 35, 49, and 61 mg/L for cow, buffalo, sheep, goat, humans, donkeys, and mares, respectively);[62,63] the levels are 5 times higher than cow's milk. The levels rise with increasing lactational days.[64] There is also seasonal variation; the levels rise during summer (227 ± 110 mg/L in summer vs 180 ± 62 mg/L and 157 ± 58 mg/L in autumn and winter, respectively).[65] The rationale for high levels of vitamin C during hot and dry seasons is unclear; it could be linked to differences in glucose metabolism in camels compared with other ruminants.

The levels of vitamins A (50–390 µg/L) and E (6.6 mg/L) are lower than in cow's milk.[62] Vitamin K content is only one-third of that in cow's milk.[58] Vitamin D (15.6 ± 2.01 ng/mL vs 1.78 ± 0.99 ng/mL) is higher.[28] Camel milk also contains high levels of carnitine (235–290 nmol/L), which is involved in fatty acid transport.[66]

Other Unique Compositions of Camel Milk

Camel milk has less lactose than cow's milk (4.47 ± 0.66 grams/100 mL vs 4.5–5 g/100 mL cow's milk).[5, 67] Camel milk generates less casomorphine, resulting in reduced intestinal motility and prolonged exposure to lactose.[40] Additionally, the higher proportion of *L*-lactate in camel milk (2.21% of total lactate) compared with cow's milk (0.02%) suggests easier absorption in the gut.[68] These differences in lactose metabolism products are likely influenced by the microflora present in camel milk with lactose being unaffected by seasonal variation.[69,68]

Camel milk contains seven oligosaccharides, surpassing cow's milk in quantity. These oligosaccharides exhibit significant prebiotic activity, inhibiting the attachment of pathogenic microorganisms to the colon and promoting the growth of beneficial *Bifidobacteria* spp.[70] Mature camel milk typically contains 0.12 gm/100 mL of oligosaccharides, higher than those in cow's milk (0.04 g/100 mL). However, this ratio is reversed in colostrum (0.20 gm% in camels vs and 0.29 gm% in cows).[71]

Camel milk is less allergenic due to the absence of beta-lactoglobulin and lower levels of beta-casein. It contains higher concentrations of lactoferrin, lysozyme, lactoperoxidase, IgG, and vitamin C compared with cow's milk, resulting in stronger antibacterial and antiviral properties.[41] Notably, it contains higher levels of lactoferrin than cow's milk (220 mg/L vs 110 mg/L) and lysozyme (288 mg/100 mL vs 13 mg/100 mL).[40]

Camel milk contains various protective proteins with antibacterial and immunological properties, such as *N*-acetyl glucose aminidase, lysozyme, lactoferrin, lactoperoxidase, and peptidoglycan recognition protein, which are effective against microbial growth.[28,71,72] Higher insulin-like proteins facilitate the absorption of nutrients, and whey proteins may protect against atopic agents.[28,72]

Chemical Composition of Camel Colostrum

The chemical composition of colostrum is shown in Table 3.

Protein

The protein content of camel colostrum ranges from 14.3 to 20.2 gm%.[73] On the 1st day of lactation, glutamic acid is the

Table 3: Mammalian colostrum (g/L; within 24 hours after birth)

	Protein (gm%; mean ± standard deviation)	*Fat (gm%)*	*Lactose (g/%)*	*IgG (g/L)*
Human	1.8 ± 0.6	3.8 ± 1.08	6 ± 1.08	2.01 ± 0.8
Dromedary camel	0.14 ± 0.3	0.01–0.17	0.26 ± 0.2	0.47 ± 0.18
Bactrian camel	0.14 ± 0.08	0.27 ± 0.12	0.43 ± 032	0.45 ± 0.26
Cow	3.8 ± 1.2	3.9 ± 1.3	1.8–2.5	1.6–3.4
Sheep	1.9 ± 0.7	6.4 ± 1.7	2.8 ± 1.5	2.4 ± 0.8
Goat	3.1 ± 2.4	4.8 ± 0.8	3.6 ± 2.3	1.9 ± 1.2
Buffalo	5.2 ± 2	8.1 ± 2.1	2.4 ± 1.1	1.2 ± 0.8

Table 4: Longitudinal changes in immunoglobulin concentrations in camel milk after birth (gm%; mean ± standard deviation)

	IgG1	*IgG2*
Day 1 after birth	38.6 ± 12.8	3.1 ± 1.2
Day 7 after birth	4.9 ± 2.2	0.4 ± 1.1
Day 14 after birth	1.3 ± 0.8	0.1 ± 0.1

primary amino acid, playing a vital role in fetal nitrogen and carbon metabolism. Proline, leucine, and aspartic acid are also present in significant amounts.[74] High concentrations of IgG, less casein than mature milk, and the absence of β-lactoglobulin impart beneficial immunomodulatory characteristics.[75] Camel colostrum closely resembles human milk, making it a promising substitute food for infants who do not have access to mother's own milk.

In the 1st 3 days after delivery, camel colostrum contains high levels of immunoglobulins (IgG_1, IgG_2, and IgG_3), with IgG_2 and IgG_3 lacking light chains. The longitudinal changes in IgG1 and IgG2 are summarized in Table 4.

In addition, there are multiple immune stimulants such as camel serum albumin, lactoferrin, and alpha-lactalbum.[75–78] In the first hour after delivery, camel colostrum contains high levels of camel albumin, as high as 20.8 grams/L, and these levels fall gradually to 10.8 grams/L over the 1st week.[79] Notably, this was a specific difference; comparison with colostrum levels of alpha-lactalbumin, at about 2.2 grams/L, did not differ between 1-humped dromedary or two-humped Bactrian camels and cows.[80]

Colostrum Lactoferrin

Colostrum from the two-hump Bactrian camels can contain more than three-times more lactoferrin than in whole milk samples.[81] Studies from Tunisia show that colostrum from the one-humped dromedary and two-humped Bactrian camels typically contain lactoferrin levels averaging 1.21 ± 0.28 grams/L, peaking at 2.3 g/L at about 48 hours post-delivery.[80] One-humped camel colostrum contains higher lactoferrin concentrations, reaching 5.1 grams/L on the 2nd day of lactation.[82] Lactoferrin content in camel milk peaks in the first 48 hours after birth and is significantly higher than bovine colostrum. The highest lactoferrin concentration in camel milk occurs at about 48 hours after birth, declining to 0.34 mg/mL after 30 days, when bovine milk contained only 0.06 mg/mL.[13]

Colostrum Insulin

The highest insulin concentration in camel colostrum was recorded at the time of delivery (1856.8 ± 804.4 µmol/mL) and levels decreased within 24 hours (367.5 ± 286.1) µmol/mL. Over the next 7 days, the levels gradually declined further to 101.3 ± 65.6 µmol/mL.[83,84]

Lipids

In the one-humped dromedary camels, the colostrum fat content at 2 hours after delivery is low at around 0.1 gm%, much lower than the typical 3 gm% levels seen in mature milk.[82] The fat content gradually increases to peak at about 7 days and then begins to decrease again. Similarly, in two-humped Bactrian camels, the fat content for the first milking ranges from 0.27 to 0.35 gm%.[85] Compared with mature milk, colostrum contains relatively less saturated FAs but higher levels of polyunsaturated FAs.[80]

Lactose

On the 1st day after delivery, colostrum contains low levels of lactose but it increases gradually over time.[81] Colostrum from one-humped dromedary camels contained ~ 2.6% lactose, double the amount found in two-humped Bactrian camels.[80] The two-humped camel colostrum contained more sialyl oligosaccharides (0.2 gm%) than in mature camel milk (0.12 gm%) or bovine colostrum.[85] The two-humped camel mammary glands, during early lactation, may synthesize high levels of sialyl oligosaccharides.[86] Given its high content of beneficial oligosaccharides, camel colostrum could be a promising option for human infant nutrition, especially as cow's milk lacks oligosaccharides. Camel colostrum could be a useful supplement with infant formula.[87]

Minerals and Vitamins

Colostrum from one-humped dromedary camels contains high concentrations of essential minerals, such as iron, zinc, and sodium at 146.5 mg, 0.2 mg, and 43.7 mg%, respectively, which are higher than in mature milk.[77] This abundance could make camel colostrum a valuable addition to gluten-free products if needed.[65] Camel colostrum is also a rich source of vitamins, including vitamin C, with concentrations ranging from 4.9 to 20.4 mg%.[88,89] Additionally, it contains significant levels of vitamins A, E, and B_1, providing 30.7 µg, 136.9 µg, and 0.7 mg%, respectively. These nutritional properties make camel colostrum an attractive option for dietary supplementation and fortification.[90] The levels of nutrients change with maturation of milk and with species.

Camel Milk as a Nutrient

Camel milk is a potentially important source of easily digestible carbohydrates, proteins, such as beta-casein, and smaller fat globules.[48,91] It is a promising alternative to infant formula considering its high β-/α-casein ratio, protective proteins, and lower propensity to activate allergic responses.

Table 5: Composition of (A) mature milk 1 week after delivery in different mammalian species; and (B) Composition of mature camel milk

(A) In mature milk (1 week after birth)

	Human	*Cow*	*Camel*
Protein (gm%)	1.1 ± 2.35	3.06 ± 0.9	2.3 ± 1.2
Fats (gm%)	3.4 ± 1.1	2.9 ± 0.6	2.6 ± 1.1
Carbohydrates (gm%)	6.2 ± 1.2	4.3 ± 1.2	4.9 ± 0.8
Immunoglobulin IgA (gm/L)	1.35 ± 0.6	0.2 ± 0.08	–
Immunoglobulin total IgG (gm/L)	0.04 ± 0.02	0.68 ± 0.08	0.82 ± 0.55
Lactoferrin (gm/L)	1.8 ± 0.2	0.08 ± 0.01	1.06 ± 0.32
Lysozyme (µgm/L)	245 ± 42	962 ± 112	112.36 ± 22

(B) Composition of mature camel milk

Content	*Composition*
Water	83–90%
Carbohydrate/Lactose	4.47 ± 0.66 gm%
Fat	3.68 ± 1 gm%
Protein	3.28 ± 0.59 gm%
Total Solids	12.2 ± 1.6 gm%
Calcium	133 ± 81 mg%
Phosphorous	56 ± 29 mg%
Magnesium	7 ± 9 mg%
Sodium	40 ± 90 mg%
Potassium	90 ± 42 mg%
Chloride	220 ± 84 mg%
Copper	459 ± 94 µg%
Zinc	256 ± 120 µg%
Iron	14.2 ± 3.6 mg%
Manganese	16.6 ± 6.2 µg%
Selenium	6.8 ± 3.8 µg%
Vitamin C	227 ± 110 mg/L

Camel milk is vital for food security in arid and semi-arid environments.[2] It is a unique source of nutrients with therapeutic benefits; we might be looking at a remarkable superfood for addressing many health concerns (Table 5). It might also offer value as a supplement.[73] Commercialization has expanded camel milk's consumption beyond arid regions, supported by numerous studies highlighting its health benefits. Further study is needed to ascertain the nutritional/therapeutic value of camel colostrum/milk.

Camel Milk as a Nutraceutical

Camel milk could be beneficial in many illnesses, across age groups.[91] Traditional beliefs/observational studies suggest the possibility of benefit in diverse conditions including paw edema; gastrointestinal disorders, such as inflammatory bowel disease, gastric ulcers, and liver disease; respiratory disorders, such as asthma, arthritides neurodevelopmental disorders, autism, and neuroinflammatory conditions.[91] Several *in vitro* and animal models have also shown benefits.[4]

In traditional medicine, camel colostrum has been used to alleviate edema in pregnant women.[29] Despite being less recognized in human food products, colostrum from one-humped dromedary camels has been shown to have potent antimicrobial activity against *Escherichia coli* and *Listeria monocytogenes*, possibly due to its high lactoferrin content.[74] This colostrum has also been used in mothers as an antihypertensive and antioxidant agent.[73] Bioactive peptides derived from colostrum proteins exhibit antioxidant, antihypertensive, opioid- and mineral-binding, growth-stimulating, and anti-inflammatory activities.[76]

Camel colostrum contains high levels of healthy FAs, such as oleic acid, vaccenic acid, and romantic acid, making it a superior dietary choice.[92] It contains lactoferrin with its antioxidant and broad-spectrum antimicrobial activity against bacteria, fungi, and viruses.[93] Hydrolyzed compounds of camel lactoferrin following gastrointestinal digestion also show potent antimicrobial peptides, enhancing food safety and nutritional value.[93] There are also biologically relevant concentrations of alpha-lactalbumin, which promote apoptosis in damaged cells and scavenging of free radicals. Nutritionally, camel colostrum offers essential amino acids, particularly tryptophan, cystine, and lysine.[41]

Administration of camel milk in animal models of cancer improved angiogenesis and reduced the cyto-/genotoxicity of agents such as cisplatin.[94] Similarly, there was benefit in models of colitis;[95] in infections such as *Salmonella enterica*,[96] and in alcohol-induced liver injury.[97,98] We still need data on the net impact, effects on various components of disease, and interaction with environmental factors such as gut microbiota. Clinical trials are needed for validation.[4,91,99]

The immunomodulatory activities of camel milk have been best studied in inflammation, diabetes, and intestinal damage.[91, 100] Its constituent mediators can alter cytokine profiles by increasing IFN-γ and Th1 responses, while simultaneously decreasing IL-4 and Th2 responses.[91] Clinical studies support its effectiveness in chronic hepatitis B and HCV by altering cytokine levels.[36] We need focused studies to confirm if camel milk or its constituents could protect against the adverse effects of infant formula and enterally administered pharmaceuticals.[101]

The mechanisms by which camel milk could exert its antioxidant effects may involve optimization of signaling pathways involving ABTS (2,2′-azino-*bis*-3-ethylbenzthiazoline-6-sulphonic acid), DPPH (1,1-diphenyl-2-picrylhydrazyl), and FRAP (ferric-reducing antioxidant power). It also induces antioxidant mediators, such as ACE, glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx).[91,102] The activity of lipid peroxides, nitric oxide, inducible nitric oxide synthase (iNOS), and caspase-3 is curtailed.[102]

Studies have shown initial improvement in autism-spectrum disorder (ASD) symptoms measured by the Childhood Autism Rating Scale (CARS) assessment[103] after feeding with camel milk for 2 weeks.[25] However, the sustainability of these benefits and their clinical relevance remain unclear.[104] Current evidence suggests that even though there might be potential benefits, it should not replace established treatment options.[25] Future studies are needed to better understand its role, effect-size, potential benefits, and the duration of impact in ASDs.[105,106]

In diabetes, several limited, relatively low-quality studies suggest camel milk may reduce fasting plasma glucose, HbA_{1c}, and daily insulin needs,[25,107] but could increase the body mass index.[108] One study hinted at reduced microalbuminuria but it lacked a control group.[109] Unlike animal studies, human trials did not show improvement in lipid levels and liver function.

The statistical comparisons were not rigorous. There is potential in managing insulin and glucose levels, and normal dietary use appears safe.[107,110,111] However, it might be too early to recommend it for clinical intervention. Further studies are needed.

Shabo et al.[112] studied infants and children with milk and other food allergies. They noted that supplementation with camel milk provided relief in symptoms within 4 days. Further validation through large-scale clinical trials is justified. Another cross-over clinical trial assessed the safety of camel milk consumption in patients allergic to cow milk protein. They concluded that camel milk was well-tolerated and safe in children who were more than 12 months in age, and viewed it as a cost-effective and palatable alternative to other formulas.[25,113] Further work is needed to confirm the benefits of camel milk in human health and elucidate the mechanisms.

Future Prospectives

Camel milk presents several challenges in processing compared with bovine milk, such as instability when processing during ultra-high temperature, reduced rennetability, formation of fragile curds, prolonged fermentation times, low thermal stability during drying, and pH-dependent solubility changes in whey proteins.[6] The composition, structure, and functional properties of camel milk proteins differ significantly from bovine milk, with lower levels of κ-casein, higher β-casein content, and absence of β-lactoglobulin.[114] Additionally, camel milk has larger casein micelles[115] and smaller fat globules.[6,73]

Camel milk shows several major differences when compared with milk from other ruminants.[114] Furthermore, there is a lack of comprehensive information on its functional properties.[116] Addressing these gaps is crucial to fully exploit camel milk's potential.[114] To overcome these challenges, several intervention strategies are recommended.[114] We need:

- Nationally and internationally accepted standards for processed camel milk and its products, including pasteurization protocols and microbiological quality standards.[114] These parameters will help ensure product safety and consistency.[117]
- Further work to enhance the nutritional quality and functional properties of camel milk products.[74] Previous work with milk from other ruminants has helped identify key nutritional components of camel milk important for infants and ways for specific modulation to preserve health benefits.[118]
- Develop tailored processing techniques to optimize product quality and value, such as purification methods that will preserve and enhance the natural attributes of camel milk.[119]
- High-throughput studies to understand camel milk-associated metabolites, microbiota, and immune factors vis-à-vis human milk and milk from other ruminants.[120–122]
- Rigorous *in vitro*, animal, and clinical studies to substantiate the therapeutic potential of camel milk products against human diseases like diabetes and autism.[104]
- Explore novel methods for camel immunization/specific vaccination to enrich milk-borne immune factors for prophylactic/therapeutic purposes. Once these constituents in hyperimmune milk are clearly characterized, later studies could focus on induction, ex vivo/synthetic development, and structural modification to increase efficacy.[123–125]

Addressing these strategies will not only help overcome current processing challenges but also promote the global acceptance and utilization of camel milk and its derivatives.[114]

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REVIEW ARTICLE

Cranial Ultrasound as an Imaging Modality in Neonatal Sepsis to Determine Involvement of the Central Nervous System

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Abstract

Background: Globally, neonatal sepsis continues to be a significant cause of neonatal morbidity and mortality. Bedside point-of-care cranial ultrasound (POCUS) can help determine whether the central nervous system (CNS) is affected. It can help evaluate meningitis, brain abscess, changes in the spinal cord, and alterations in cerebral blood flow; it can even provide some clues for early identification of fungal and viral infections. This information can aid in appropriate management.

Methods: A comprehensive literature search was conducted to review hallmark POCUS findings in neonatal sepsis with CNS involvement. Further inputs were gathered on understanding the role of these findings in prognosticating and defining the duration of management.

Results: The review focused on the classical findings seen on cranial ultrasound, with meningitis in the cerebrum and spinal cord. The complications of meningitis, like ventriculitis, cerebral abscess, and cerebral thrombosis along with other fungal and perinatal infections with their ultrasound findings have been highlighted in this review article.

Conclusion: POCUS is a useful bedside screening tool for the diagnosis and management of neonates with meningitis and its complications. Its ease of usage, with safety, and a lesser turnaround time make ultrasound superior to other imaging techniques in neonatal infections.

Keywords: Brain abscess, Central nervous system, Cranial ultrasound, Fungal infections, Meningitis, Neonates, Point-of-care ultrasound, Sepsis, Viral infections.

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Key Points

- Point-of-care cranial ultrasound (POCUS) is a useful, low-cost tool with its ease of accessibility, absence of ionizing radiation, and no need for sedation; it is increasingly being used as a first-line imaging technique for the evaluation of neonatal infections.
- POCUS can help identify neonatal infections with major brain involvement. However, it may not be useful for predicting long-term outcomes.
- POCUS can detect cerebral calcifications and macroscopic anomalies, which helps in the diagnosis of intrauterine/early neonatal infections.
- Even though magnetic resonance imaging remains the gold-standard for diagnosing meningitis, POCUS is a useful first-line imaging modality for diagnosis, evaluation, and monitoring for complications in neonatal meningitis.
- Brain abscess may show many sonographic features similar to those seen in ischemic lesions or hematomas, but a combination of clinical monitoring and serial imaging can help differentiate between the two.

Introduction

Neonatal sepsis with meningitis is one of the most common causes of serious neonatal morbidity and mortality all over the world.[1–3] In developed countries, the recorded incidence of culture-positive neonatal meningitis is approximately 0.3 per 1,000 live births.[4] However, this may be an underestimation due to the variability of obtaining cerebrospinal fluid (CSF) in evaluating late-onset sepsis (LOS). The incidence in tropical/peri-equatorial countries is likely to

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be even higher because of more frequent gram-negative infections. Complications of meningitis, such as ventriculitis, hydrocephalus, extra-axial fluid collections, cerebritis, and brain abscess can further accentuate the severity of illness in these patients.[5] A quick bedside diagnosis, prompt identification of complications, and initiation of medications can improve the neurological outcome of these neonates and reduce mortality.[6]

POCUS is a useful, low-cost tool that is now increasingly useful with its ease of accessibility, absence of ionizing radiation, and the need for sedation.[5] It is highly useful for the identification of infected newborns with major brain involvement, even though it might be less reliable in predicting long-term neurodevelopmental outcomes than magnetic resonance imaging (MRI).

In this review, we discuss the utility of cranial ultrasound in neonates with sepsis including meningitis, ventriculitis, postinfective ventricular dilatation, findings seen in fungal and viral infections of the brain, and in brain abscess. We also have briefly discussed the utility of color doppler interrogation in these neonates. The anterior Fontanelle is used as an acoustic window, with the infant's head in midline supine position. Standard views are obtained in the coronal and sagittal planes. Additional views like the temporal and mastoid views could be obtained using other fontanelles as acoustic windows.

Meningitis

Meningitis, or inflammation of the meninges, is a life-threatening condition in neonates. Bacterial infections are responsible for the highest global burden.[7] The most-frequent etiological agents are *Group B streptococci*, *gram-negative bacilli*, and *Listeria monocytogenes*.[8] The diagnosis of meningitis can sometimes be challenging when the CSF might not show definitive abnormalities. Early diagnosis and appropriate treatment can improve the prognosis for these patients.[9] Magnetic resonance imaging remains the gold-standard imaging modality for diagnosing meningitis but, many of these critically-ill infants are not stable enough to withstand prolonged imaging or transport to radiology units in hospitals.[10] Hence, POCUS can be a useful first-line imaging modality in these patients for diagnosis, evaluation of extent/severity of infection, and assessment of response to treatment. The fact that it allows real-time assessment at the beside and without any radiation exposure make it further attractive.[11] Cranial US abnormalities have been noted in around 65–80% of infants in the acute phase of meningitis; the numbers might be higher in many specific infections and with a prolonged or severe clinical course.[11–13]

Littwin et al.[10] categorized cranial US findings in neonates with meningitis based on topographical localization (Table 1). Sonographic changes may be observed on the surface of the brain, the ventricular system, brain parenchyma, and the lumbosacral segment of the vertebral canal.

Changes on the Surface of the Brain

Meningeal Thickening and Widening of the Brain Sulci

Accumulation of inflammatory exudates is seen as enhanced echogenicity of the leptomeninges in thickened cerebral gyri and sulci (Fig. 1).[5,10] Meninges are defined as thickened when these measure more than 1.3 mm on the gyral surface or 2 mm in a sulcus.[14] Enhanced echogenicity of gyri and widening of brain sulci can be seen in 26–83% of cases of meningitis; these are the earliest and most frequentlyseen changes.[11,12,15–18] Although these changes are common, these do not have significant prognostic value.[11]

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Prominence of Cortical Vessels

Superficial cerebral veins, superior sagittal sinus, perforating vessels, and blood vessels within the pia-arachnoid may get dilated and appear prominent on color doppler sonography. These results reflect meningeal inflammation due to infection (Fig. 2).[10,11]

Extra-axial Fluid Collection

These fluid collections are detected more easily using high-frequency ultrasound probes.[19] These can be seen in 5–33% of patients with meningitis, and generally represent reactive effusions with no prognostic implications.[15,18–20] In the presence of effusions, the usually non-visible gyral surface on the convexities of the brain becomes noticeable as the brain is displaced away from the vault (Fig. 3A).[11] There is often an associated midline shift and chinking of the ventricles due to increased intracranial pressure. Empyema, a rare complication, should be suspected if the extra-axial fluid shows internal echoes, particularly in a clinical setting where a critically-ill infant shows neurological deficits and refractory seizures (Fig. 3B).[21–24] Analysis of the aspirated fluid from the collection may be diagnostic.[24] Color doppler may give clues regarding the location of the extra-axial fluid collection(s).[25,26] Subarachnoid fluid can be seen surrounding the cortical vessels, unlike subdural fluid collections that compress the vessels along the surface of the brain.

Cerebral Thrombosis

Meningitis is a risk factor for neonatal cerebral venous sinus thrombosis.[27] In affected sinuses, absent or decreased blood flow on color doppler and hypoechoic contents may be a clue to the presence of thrombosis.[28,29]

Cerebral Ventricular Findings

Ventricular Dilatation

Ventricular enlargement above the 97th centile for gestational age has been noted in up to 14–65% cases of bacterial meningitis.[12,13,16–18,20,30–32] In the acute phase, increased production of CSF (due to ependymitis and choroid plexitis) and decreased absorption (due to arachnoiditis) may lead to increased intraventricular pressure and ventriculomegaly. With the progression of infection and associated inflammation, fibroglial elements with leukocyte infiltration may form septations and obstruct CSF flow at different levels, such as at the aqueduct of Sylvius and/or the foramina of Luschka and Magendie, to result in a non-communicating hydrocephalus.[33] During the chronic phases, *ex vacuo* ventriculomegaly may occur with normal ventricular pressures due to the loss of brain parenchyma. Unlike infants with hydrocephalus due to increased intraventricular pressure that

manifests with macrocephaly and a bulging anterior fontanelle (AF). These neonates progress to develop microcephaly with a sunken AF. Figure 4 shows images from two neonates showing gross ventriculomegaly due to *gram-negative* meningitis. To evaluate the need for intervention, serial sonographic measurements of ventricular size are superior to measurements of head circumference or the assessment of clinical symptoms of raised intracranial pressure (ICP).[34,35] Sonographic measurement of the lateral ventricles is also a sensitive tool to detect *ex vacuo* ventriculomegaly in preterm infants due to the loss of periventricular white matter.

The most frequently performed measurements include:[30,36,37]

- Ventricular index (VI), defined as the distance between the falx and the lateral wall (Fig. 5A) of the anterior horn of the lateral ventricles in the coronal plane. Although the VI may begin to increase with the first rise in intracranial pressure (ICP) in some infants, it might not increase in others with mild ventricular dilation until they develop severe hydrocephalus.[38–40]
- Anterior horn width (AHW) is defined as the diagonal width of the anterior horn (Fig. 5B) measured at its widest point in the coronal plane. Govaert and de Vries LS[41] suggested that AHW >6 mm can be associated with ventricular ballooning, and need for treatment.
- Thalamo-occipital distance (TOD) is defined as the distance between the outermost point of the thalamus (at its junction with the choroid plexus) and the outermost part of the occipital horn in the parasagittal plane (Fig. 5C). In some infants, measurement of the TOD can be challenging due to difficulties in clearly defining the occipital border. The occipital horn may show rapid enlargement due to post-hemorrhagic or postinfective ventricular dilatation.[42,43] Isolated occipital horn dilatation may be associated with signs of increased ICP and signify an urgent need for intervention.
- Dilatation of the 3rd and 4th ventricles (Figs 5D and E), in association with the enlarged lateral ventricles, may help in differentiating between communicating and non-communicating hydrocephalus.[36] Enlargement of the 3rd and 4th ventricles may also help in distinguishing postinfective ventriculomegaly due to ex-vacuo dilatation.[44] Isolated dilatation of the 3rd and/or 4th ventricles can be associated with posterior fossa hemorrhage. Dimensions of these ventricles can be difficult as reference values are scarce.[36,37] Imaging of the 3rd ventricle in the coronal plane can be difficult unless it is dilated.[45] The 4th ventricle can differ in shape between subjects, and consequently, there may be some interobserver variability.[36]

Ventriculitis

Ventriculitis is seen in 7–23% of cases of meningitis.[12,20,46–48] This condition is diagnosed when sonograms show (A) internal echoes within the ventricular cavity; and (B) irregularity and thickening of the ependymal lining and choroid plexus with increased echogenicity (Fig. 6A). In long-standing disease, fibrous septa (Figs 6B and C) may be seen along the ventricular pathway, resulting in intraventricular compartmentalization, intraventricular cysts, and obstructive hydrocephalus.[5]

Changes in the Brain Parenchyma

Cerebral Edema

Moderate-severe cerebral edema manifests with effacement of sulci and gyri, increased echogenicity of brain parenchyma, and chinking of ventricles and other cisternal structures (Fig. 7).[18,49,50] Increased ICP associated with cerebral edema may increase the resistive index (RI) in cerebral vessels such as the anterior cerebral artery (ACA).[10,11] Patel et al.[12] showed that increased parenchymal echogenicity with chinked, small-size ventricles suggested cerebral edema in 30.7% of infants with meningitis. In another study, Chowdhary et al.[51] noted similar findings in 65% cases.

Focal Brain Parenchymal Lesions

Focal lesions with altered or increased echogenicity on cranial US may indicate infarction, cerebritis, abscess, or hemorrhage.[5,11,51] These changes may be noted in 12–65% of infants with meningitis (Fig. 8).[12,15,18,51] Presence of parenchymal lesions are associated with poor prognosis and need follow-up.[11,15]

Changes in Spinal Cord

The vertebral arch and spinous processes are not fully ossified until 12 months of age, and consequently, provide an acoustic window to visualize the spine. Presence of echogenic debris in the subarachnoid space in the thoracolumbar spine seen, in infants with intracranial hemorrhage or bacterial meningitis, may be associated with a higher risk of progressive hydrocephalus.[52]

Brain Abscess

Cerebral abscess, a rare intracranial infection, is associated with high mortality and long-term neurological deficits.[53,54] In temperate climates, these are caused most often by gram-negative organisms

Table 1: Cranial ultrasound findings in neonatal meningitis

Finding	*Pathophysiology*	*Incidence*
Meningeal gyral thickening (>1.3 mm) and widened brain sulci (>2 mm)	Accumulation of inflammatory exudates	26–83%[11,12,15–18]
Prominence of cortical blood vessels	Inflammatory reaction to infection	
Extra-axial fluid collection	Reactive effusion or empyema	5–33%[15–18,20]
Cerebral thrombosis	Pathophysiology unknown, likely due to direct bacterial invasion, activation of coagulation, post-infectious immunoglobulin deposition	1%
Ventricular dilatation	*Acute state* – ependymitis, choroid plexitis, arachnoiditis, leading to obstruction along the ventricular system *Chronic state* – Hydrocephalus ex-vacuo	14–65%[12,13,16–18,20,31,32]
Ventriculitis	Intraventricular inflammatory exudates with ependymitis	7–23%[12,20,46–49]
Cerebral edema	Cytotoxic and interstitial edema due to increased permeability of blood-brain barrier and inflammatory response	30–65%[12,51]
Focal brain parenchymal lesions	Occur due to infarction, cerebritis, abscess or hemorrhage	12–65%[12,15,18,51]

such as *Proteus* and *Citrobacter*; and gram-positive *Streptococcus* spp. In tropical/peri-equatorial countries such as India, *Klebsiella* is a frequent causative agent.[53,55,56] Other organisms may include *Serratia*, *Neisseria meningitis*, and gram-positive organisms such as *Staphylococcus aureus*.[55,57,58] Apart from these bacteria, fungi such as *Candida* are also seen frequently.[59,60]

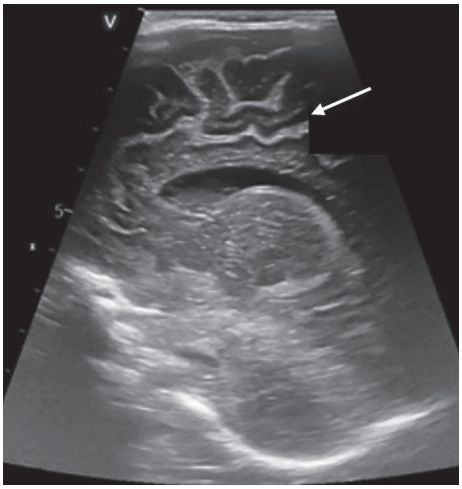
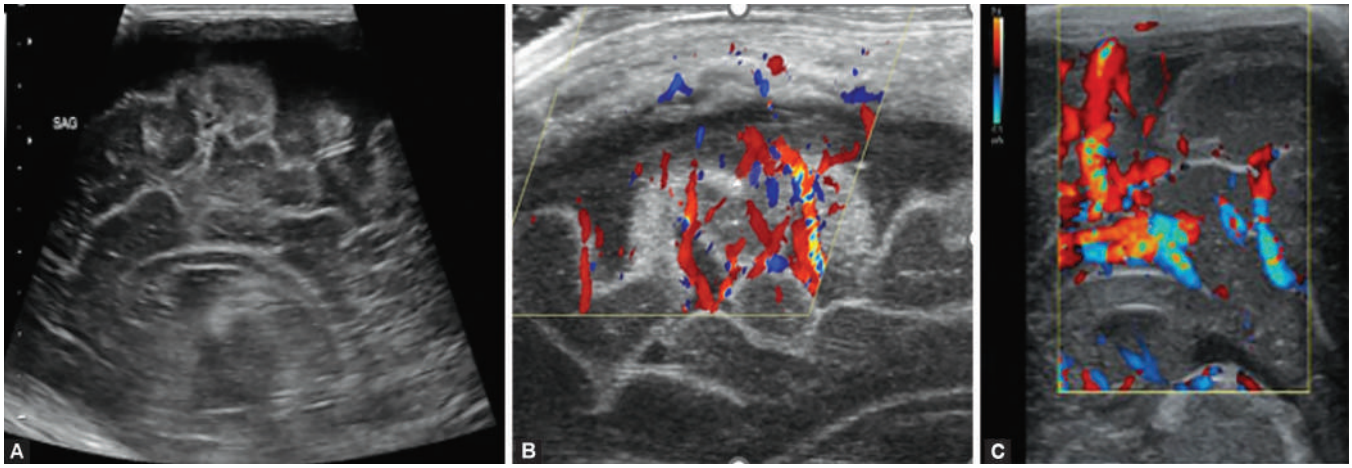
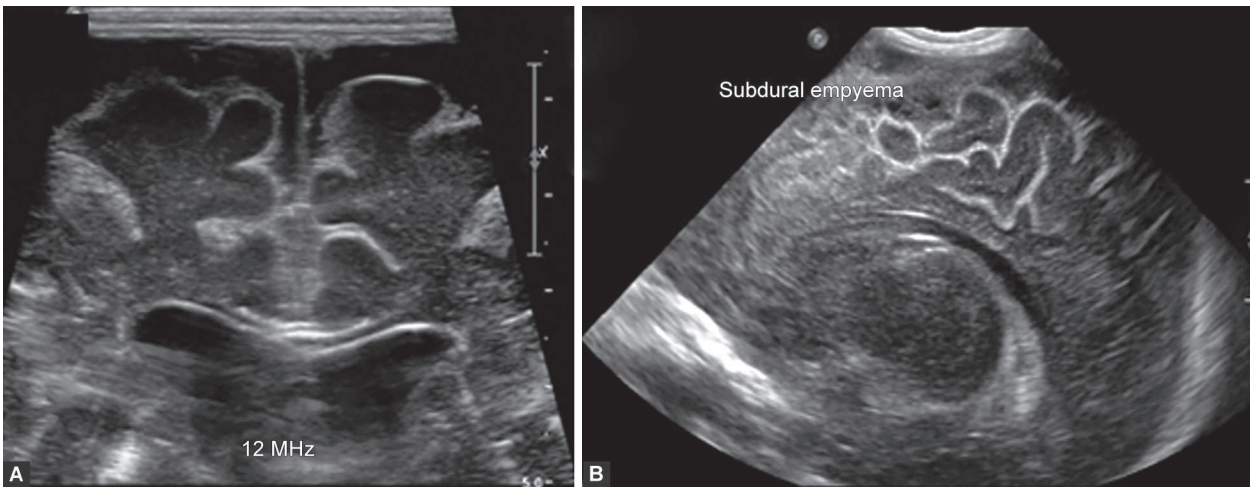


Fig. 1: Parasagittal view which shows echogenic sulci in a case of meningitis (white arrow)

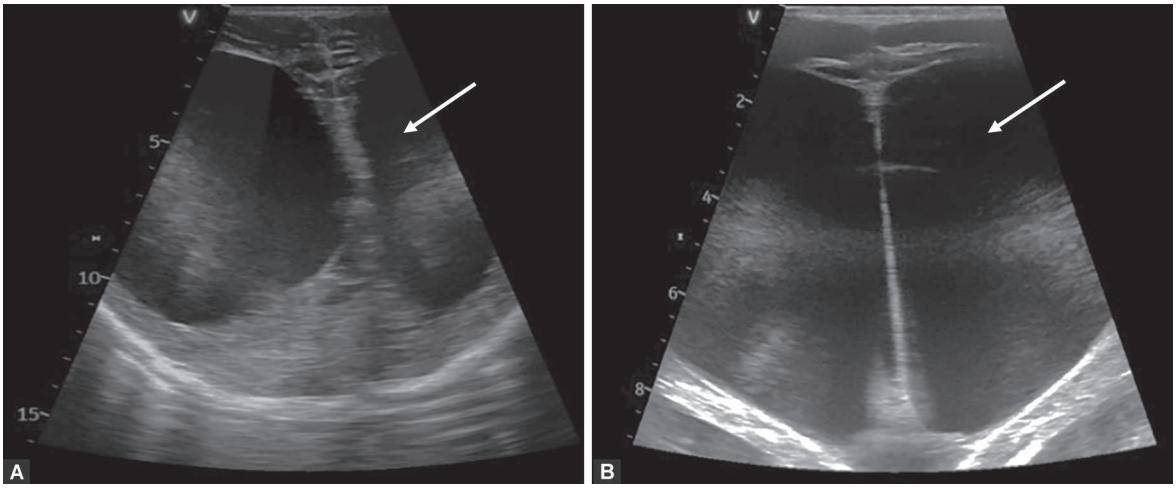
The most-frequent site of cerebral abscess is the frontal lobe.[54,56] A localized area of cerebritis can progress to form a pustular cavity, which then gets covered by a capsule. In many cases, it gets complicated to develop ventriculitis through ventricular connections, followed by hydrocephalus.[53] On the cranial US, the appearance of an abscess depends on the stage of infection. Initially, it may appear as increased echogenicity with poorly-delineated margins and increased vascularity on color doppler. As it matures, the abscess becomes a well circumscribed lesion with increased wall echogenicity (Fig. 9). This may further progress to cavitation with marked peripheral hyperaemia.[11] Aspergillus brain abscess may lead to hemorrhagic brain abscess, which may mimic post-ischemic or post-hypoxic intracranial hemorrhage.[61] In addition to a primary abscess, many cases show multiple secondary abscess.[53,56] Secondary complications include increased ICP with midline shift, ventriculitis, and hydrocephalus.[53,57] To diagnose brain abscess, sonography is usually performed via the anterior fontanelle. Cerebellar abscess may be imaged better using the posterior fossa approach through mastoid fontanelle.[62–64]



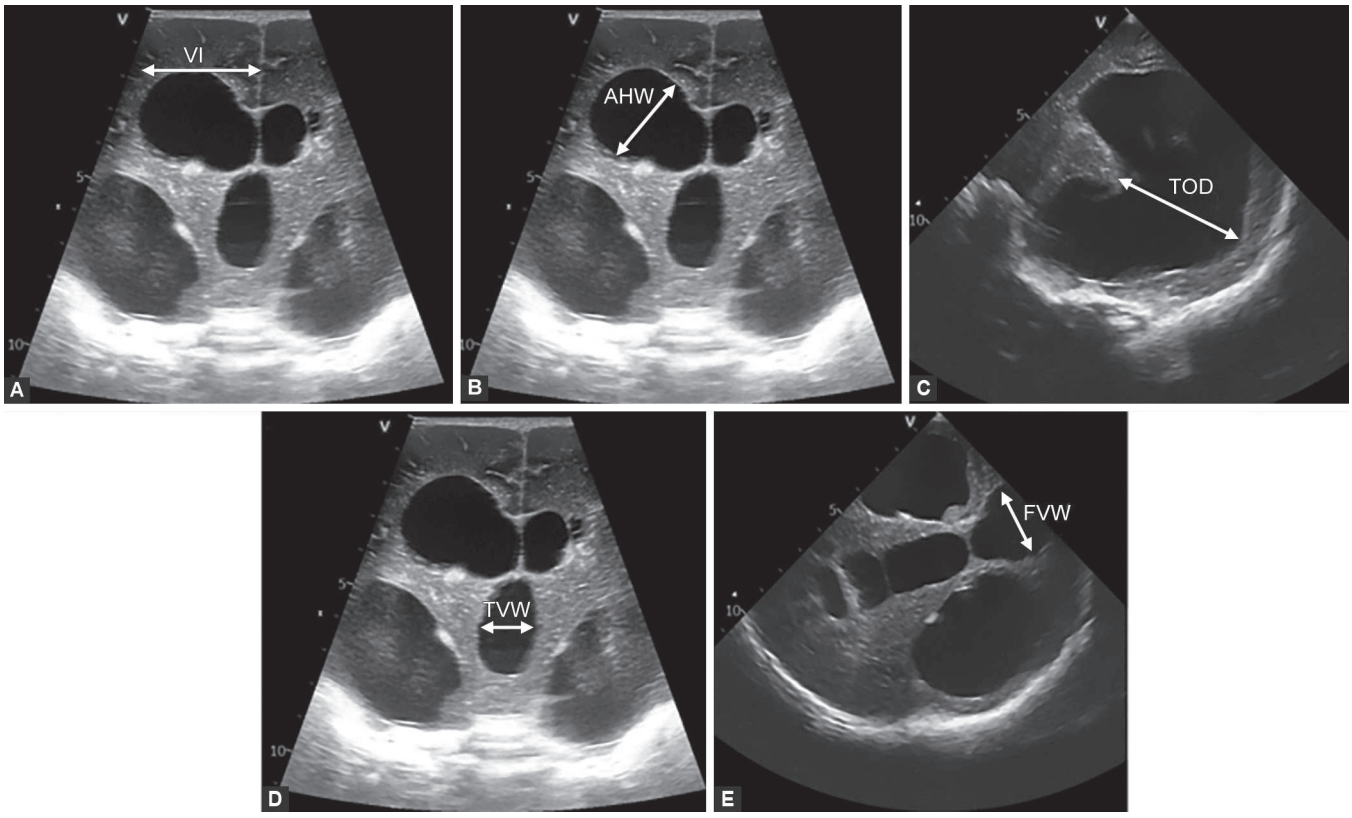
Figs 2A to C: (A and B) Prominence of the cortical blood vessels in a case of *Klebsiella* meningitis (A grey scale, B color); (C) Increased vascularity of cortical vessels



Figs 3A and B: (A) Increased bifrontal extra-axial fluid collection (EAFC); (B) Subdural empyema



Figs 4A and B: (A) Term neonate presented in the third week of life with multiple seizures, and CSF revealed *E. coli* meningitis. Coronal view revealed gross hydrocephalus (arrow shows enlarged ventricles); (B) Coronal US view of a term neonate who presented in the second week of life with *Klebsiella* meningitis and gross hydrocephalus

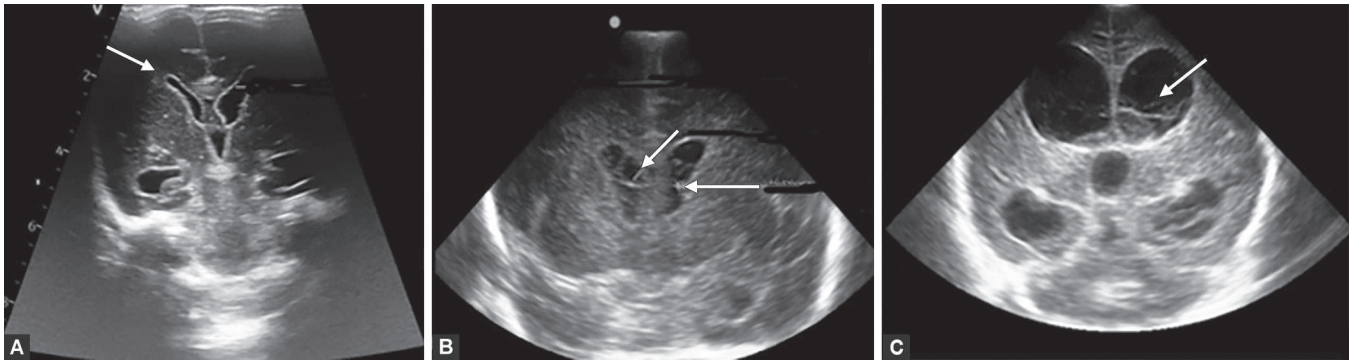


Figs 5A to E: Postinfective ventricular dilatation. (A) Ventricular index (VI); (B) Anterior horn width (AHW); (C) Thalamo-occipital distance (TOD); (D) Third ventricle width (TVW); (E) Fourth ventricle width (FVW)

On sonographic evaluation, abscess may resemble ischemic lesions or hematomas.[54,57] In such situations, a combination of clinical assessment and serial sonographic imaging can be helpful.[65] Magnetic resonance imaging (MRI) has the highest sensitivity and specificity to diagnose brain abscess, but logistical difficulties related to cost, availability, need for sedation, and transport to the radiology sections in the hospital are deterrents.[55] Computed tomography (CT) with contrast can help in diagnosing abscess; ultra-fast imaging is an advantage, but the risk of radiation exposure in serial imaging causes concern. Documentation of the increasing size of the abscess can aid decision making about interventions such as guided radiological aspiration or surgical evacuation/resection. Ultrasound guided aspiration of abscess(es) has been used for the treatment of neonatal abscess with good results.[57,65]

Cerebral Doppler Changes in Sepsis

In neonatal sepsis, doppler evaluation of cerebral blood flow (CBF) has shown conflicting results. Early-onset neonatal sepsis may show decreased resistance and increased CBF.[66–69] Yengkhom



Figs 6A to C: (A) Echogenic lining of the ventricles suggestive of ventriculitis; (B and C) White arrows indicate fibrous septa and echogenic débris in the ventricles

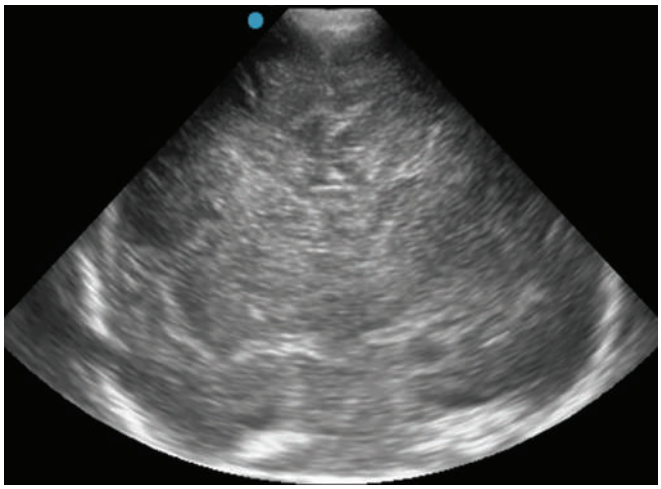


Fig. 7: Cerebral edema showing effacement of sulci and gyri, increased echogenicity of brain parenchyma and slit-like ventricles

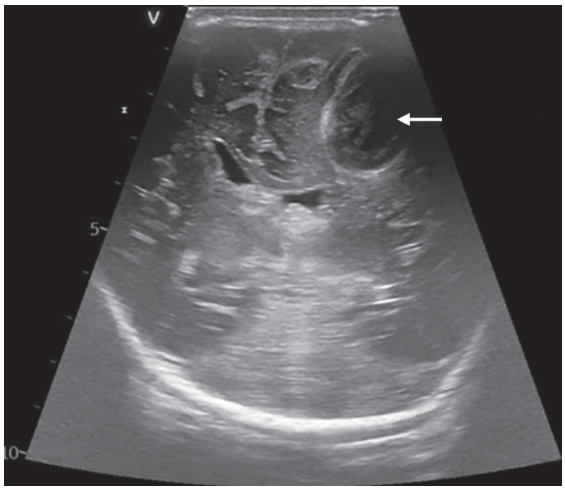


Fig. 8: Focal parenchymal lesion (white arrow) with altered echogenicity causing a midline shift

et al.[70] studied late-onset neonatal sepsis; they noted a high resistive index (RI), indicating decreased CBF. However, not many studies have been done to describe the effect of intracranial infections on RI and CBF. In intracranial infections, increased intracranial pressure due to cerebral edema, postinfective hydrocephalus, or extra-axial fluid collections may lead to increased pulsatility of arterial blood flow, causing elevated RI.[10,11,71] The effect of meningitis on RI needs further research.

Fungal Infections of the Brain

In infants and children, fungal brain abscess constitute almost 20% cases but show nearly 80% mortality.[72] Most of these intracranial abscess occur in critically-ill/preterm neonates who have had a prolonged stay in the intensive care units, where they have received assisted ventilation and long courses of antibiotics. Systemic disseminated candidiasis with such abscess is associated with mortality rates nearing 30–54%. Brain and kidney are two frequently-affected organs; *Candida albicans* and *Candida parapsilosis* have been recognized most often in these infections.[73,74]

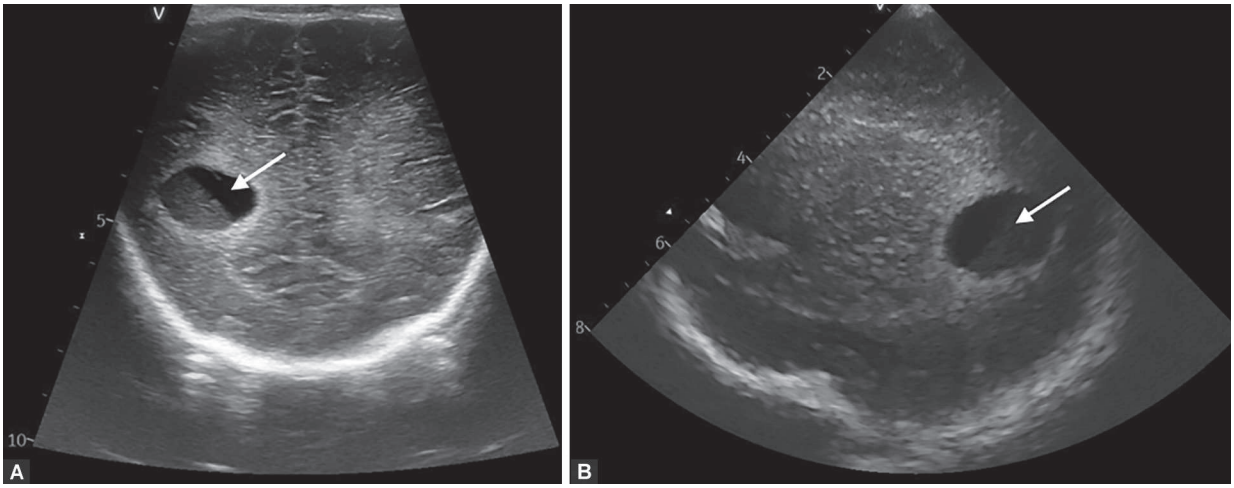
The brain lesions associated with fungal infections include enlarged ventricles, ventriculitis with ventricular septations, echogenic débris in the ventricles, hydrocephalus, periventricular cavitation, diffusely decreased parenchymal echogenicity, focal infarctions, cerebritis, abscess, and granulomas. Cerebral micro-abscess has been associated most frequently with *C. albicans* infections in preterm neonates.[75,76] Macroabscess (Fig. 10) associated with non-caseating granulomas have also been seen. The sonographic appearance of fungal ventriculitis can potentially be confused with intraventricular hemorrhage (IVH), which may be a concurrent sonographic finding in a premature infant. The timeline of disease and its progression allows distinction between these entities (Fig. 11).

There is insufficient evidence to recommend exact duration of antifungals for treating fungal abscess which is usually 6–8 weeks depending on the organism and response.[58] The location of micro-abscess is reportedly in the frontal lobe, bilateral cerebral hemispheres and periventricular regions.[59]

TORCH Infections and the Neonatal Brain

Intrauterine and perinatal congenital viral infections can affect the fetal/neonatal brain and manifest in various clinical manifestations. Therefore, neonatal neuroimaging including cranial US, is important for clinical assessment and prognostication.

Cranial US can detect cerebral calcifications and possible macroscopic anomalies. It is the primary diagnostic tool for TORCH infection [toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex, and other organisms, including syphilis, parvovirus B19,



Figs 9A and B: Solitary cerebral abscess seen in the (A) coronal; and (B) sagittal view

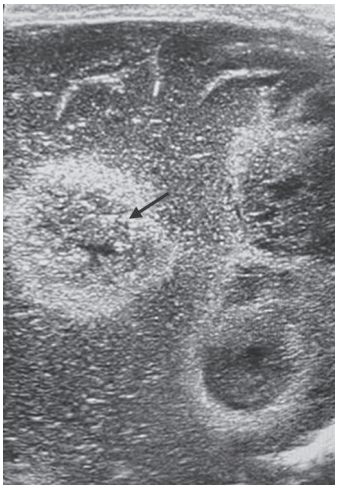


Fig. 10: Fungal macroabscess (arrow) in the brain

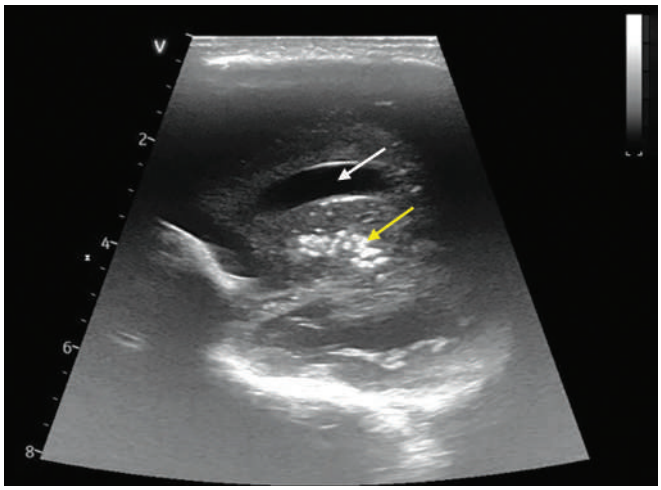


Fig. 11: Hydrocephalus (white arrow) and calcifications (yellow arrow) in congenital toxoplasmosis

and Varicella zoster. Zika virus is considered the most recent member of TORCH infections suspected *in utero* or after birth.[77]

Even though it is the primary screening technique, there are limitations due to its moderate sensitivity for subtle cerebral parenchymal changes.

Infections occurring in the early intrauterine period can manifest with anomalies such as microcephaly, hydrocephalus, parenchymal calcifications, and white matter abnormalities.[78,79] Most TORCH infections, particularly CMV and rubella virus infections (Table 2),[80] manifest with white matter changes. Toxoplasmosis frequently affects basal ganglia, thalami, cerebral cortex, and periventricular tissue.

CMV Infections

Pathological brain ultrasound findings in congenital CMV infections include intracranial calcifications, ventriculomegaly (axial diameter >10 mm across the atrium, at the level of the posterior horn), germinolytic cysts, and/or lenticulostriate vasculopathy (LSV; (Fig. 12). As a predictor of neurological sequelae in CMV infections, cranial US has a sensitivity of 83.3%, (90% CI, 58–100), specificity of 44.4% (90% CI, 18.7–70.2), positive predictive value of 50% (90% CI, 25.6–74.4), and a negative predictive value of 80% (90% CI, 50.2–100).[81]

Alarcón et al.[82] devised a cranial US scoring system (Table 3), which suggests that transfontanelle ultrasound is comparable to CT scans in its ability to detect most cerebral lesions caused by CMV. The Noyola classification, originally designed for CT scan scoring in CMV infections, is also applicable to transfontanellar ultrasound findings.[83] The association of LSV with congenital CMV infections is still inconclusive.[84] If the features of LSV are not detectable at birth but can be seen at term-equivalent age, the possibility of postnatal CMV infection should be considered.[85] Some cranial US findings in CMV infection, like cerebellar hypoplasia and migration disorders like polymicrogyria (PMG), are likely to be missed.[86,87]

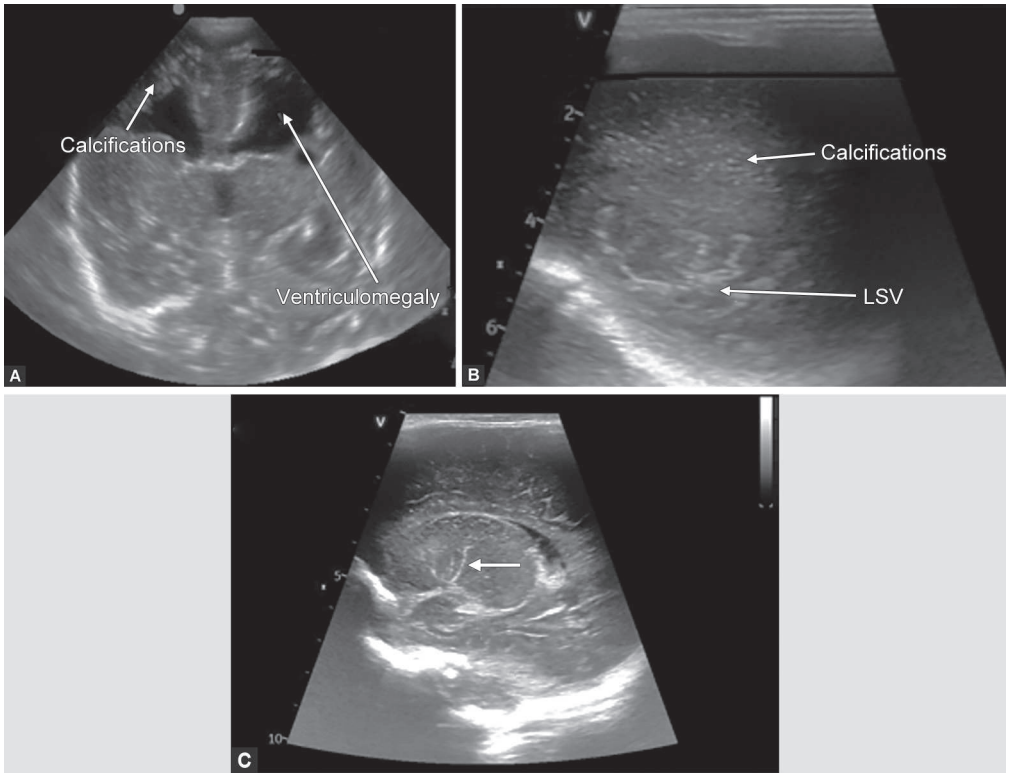
Other Viral Infections

In Herpes simplex infections of the neonatal brain, the cranial US may show abnormalities in the white matter and central grey nuclei. However, many abnormalities of the cortex, cerebellum, and brain stem may be missed.[88–90]

We still have limited information about parvoviral infections of the brain. Cranial US may show diffuse echogenicity in the periventricular and the deep white matter. In Rotaviral infections

Table 2: Main sonographic features of TORCH infections[80]

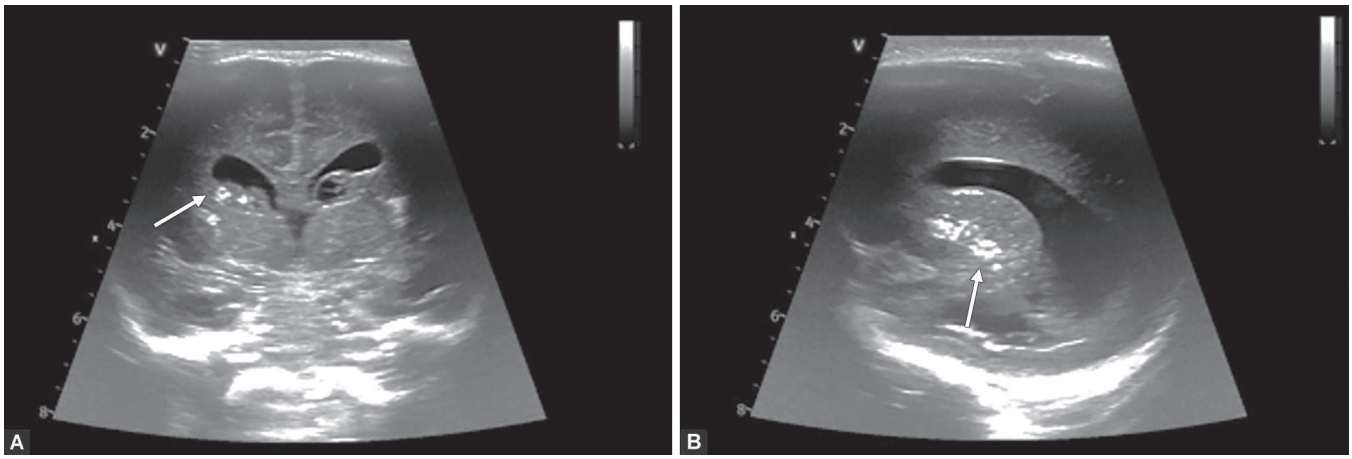
Organism	*Calcifications*	*Ventricles*	*Cortex*	*White matter*	*Other*
Cytomegalovirus (CMV)	Periventricular distribution, punctate	Ventriculomegaly	Polymicrogyria, lissencephaly, schizencephaly	Periventricular delayed myelination, germinolytic cysts, temporal cysts	Hemorrhage (rare). Cerebellar hypoplasia
Toxoplasmosis (Fig. 11)	Extensive, most commonly in basal ganglia, thalami, cerebral cortex, and periventricular tissue	Hydrocephalus, hydranencephaly (rare)	Microcephaly, macrocephaly, porencephaly	Microcephaly, macrocephaly, porencephaly	Aqueduct occlusion/stenosis secondary to epididymitis
Herpes simplex virus (HSV)	Less common	Ventriculomegaly, hydranencephaly	Microcephaly, porencephaly	Microcephaly, porencephaly	Corpus callosum agenesis, microphthalmia, meningeal involvement
Rubella (Fig. 13)	Basal ganglia and periventricular	Ventriculomegaly	Polymicrogyria	Extensive multifocal white matter hyperintensities, mainly in the frontal lobes	Subependymal cysts, lenticulostriate vasculopathy, myelination delay, cerebellar hypoplasia
Parvovirus	Less common	Ventricular dilatation	Ischemic and hemorrhagic strokes, polymicrogyria (rare), heterotopia (rare)	Ischemic and hemorrhagic stroke	Cerebral vasculitis, hydrops
SARS-COVID19		Intraventricular hemorrhage	Parenchymal hemorrhage, cerebral ischemia	Periventricular leukomalacia, parenchymal hemorrhage, cerebral ischemia	Cerebral vein thrombosis
Zika virus	Corticomedullary junction in frontal and parietal lobes, less frequently in the thalamus, basal ganglia, cortex, and periventricular regions	Ventriculomegaly	Microcephaly, polymicrogyria, gyral simplification, pachygyria-lissencephaly, opercular dysplasia, heterotopia	Microcephaly, delayed myelination, dysmyelination, hypoplasia of corticospinal tracts	Pseudocysts in the occipital horns, asymmetrical microphthalmia, cataracts, optic nerve atrophy, coloboma, lens defects, herniation of the orbital fat into the cranial vault, thin spinal cord



Figs 12A to C: Congenital CMV infection of the neonatal brain: (A) Coronal image shows evidence of ventriculomegaly and calcifications; (B) Sagittal image shows calcifications; and (C) Lenticulostriate vasculopathy (LSV) in parasagittal view (white arrow)

Table 3: Postnatal cranial ultrasound scoring system for congenital CMV infections[82]

Grade I	None of the findings below
Grade II	Single punctate periventricular calcification, lenticulostriate vasculopathy, caudothalamic germinolysis, ventriculomegaly (excluding severe)
Grade III	Multiple discrete periventricular calcifications, paraventricular germinolytic cysts, severe ventriculomegaly
Grade IV	Extensive calcification, brain atrophy

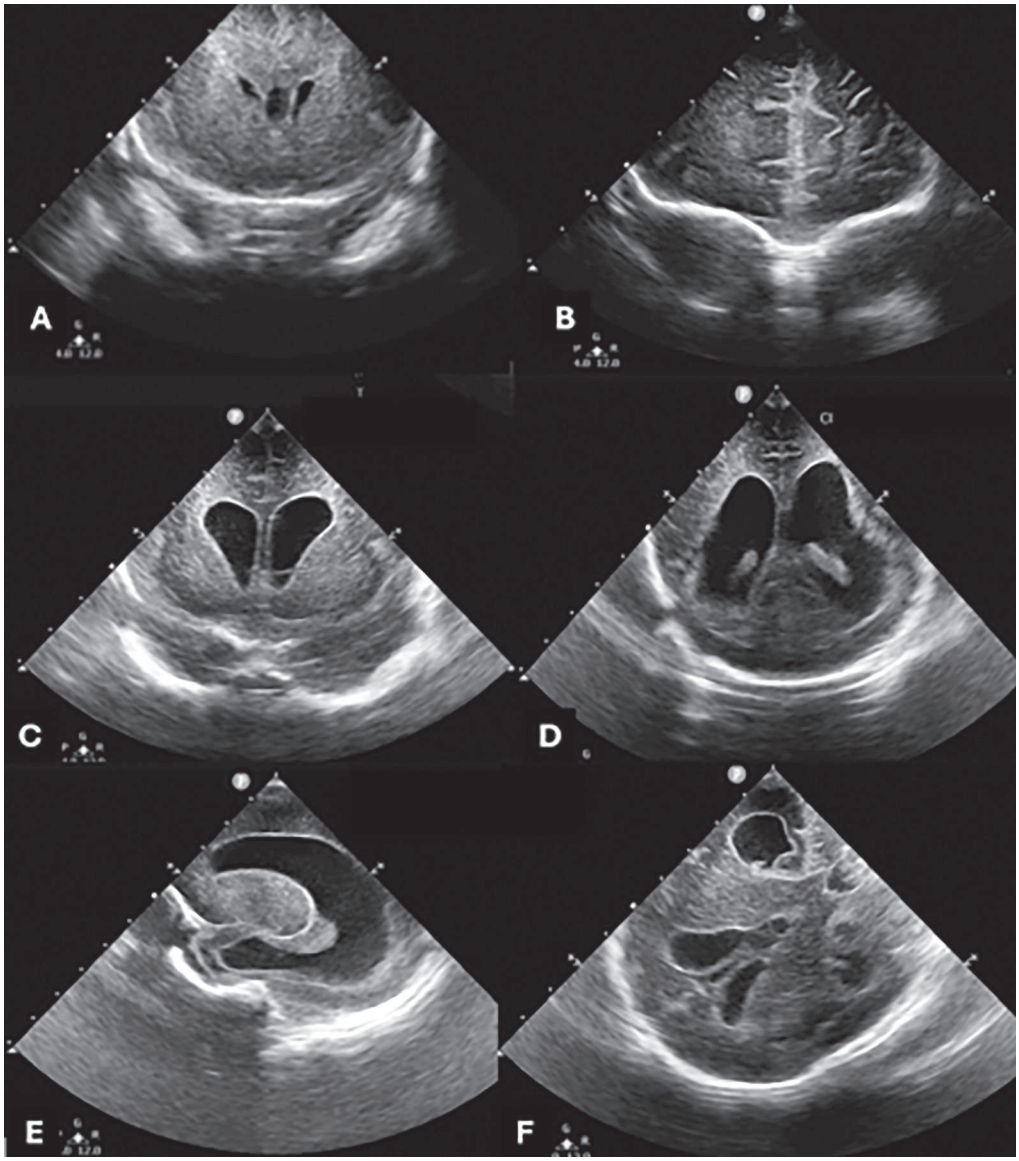


Figs 13A and B: Term neonate born with low-birth-weight presented on postnatal day 5 with seizures, and was diagnosed with congenital rubella infection. Calcifications (arrows) seen in (A) coronal; and (B) sagittal view

of the brain, from 24 to 48 hours after the onset of symptoms, the cranial US may show a diffuse increase in white matter echogenicity (Fig. 13).[91]

Conclusion

Cranial US is emerging as a valuable tool for screening, identification, and assessment of the progression of cranial



Figs 14A to F: Postinfective ventricular dilatation

abnormalities in preterm and full-term neonates with infections. A wide range of pathologies of bacterial, viral, and fungal infective etiology can be confirmed on the cranial US for timely institution of treatment. There are limitations in diagnosing parenchymal brain involvement. However, the ease of use and portability of the cranial US make it an ideal point-of-care tool for premature/critically ill neonates.

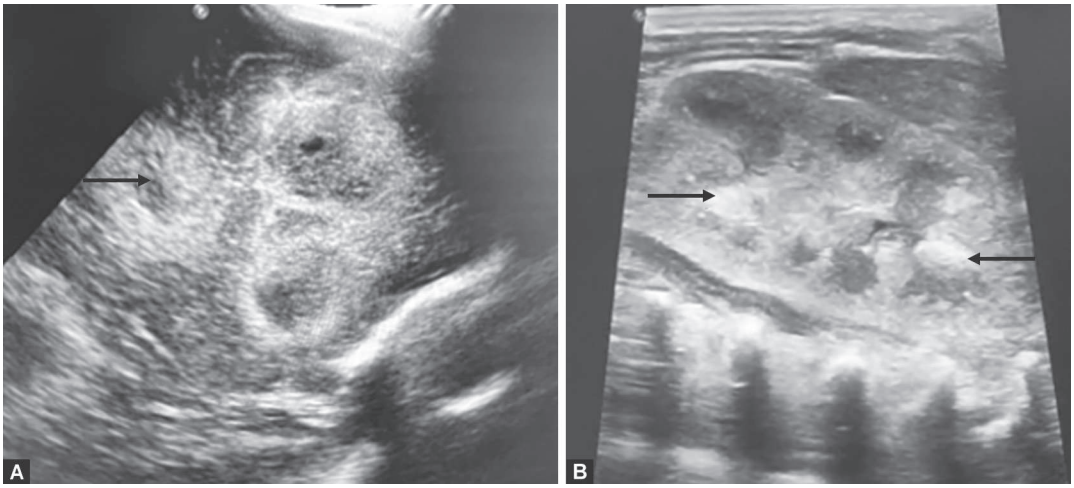
Case Study 1

A preterm neonate (gestational age 28^{+2} weeks) developed tachycardia and feed intolerance on postnatal day 15. Blood cultures grew *Acinetobacter spp*. Coronal (A–D), Sagittal (E) and mastoid (F) views (Fig. 14) reveal postinfective ventricular dilatation:

- Normal-sized ventricles (Day 15).
- Thick exudates seen in the sulcal spaces (Day 15).
- Dilated lateral ventricles with ventriculitis (Day 25).
- Dilatation of occipital horns of lateral ventricles (Day 25).
- Increase in the thalamo-occipital distance (Day 25).
- Dilatation of the 3rd and 4th ventricles (Day 28).

Case Study 2

A full-term neonate was admitted on postnatal day 15 with abdominal distension and bilious vomiting. Laparotomy revealed multiple colonic perforations. The baby had received cephalosporins for 10 days prior to surgery. CSF analysis done on postnatal day 18 revealed polymorphonuclear leukocytosis. Cranial US (Fig. 15A) showed three ill-defined hyperechoic shadows in the frontoparietal region. A renal US revealed fungal balls in the kidney (black arrows; Fig. 15B). Blood and CSF cultures grew *Candida albicans*. The infant was treated with antifungals for 8 weeks and discharged home thereafter.



Figs 15A and B: Disseminated fungal infection: (A) Cranial ultrasound; (B) Renal scan (black arrows)

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REVIEW ARTICLE

A Primer on Epigenetic Changes: The More We Know, the More We Find in Fetuses and Infants

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Abstract

Epigenetics is the study of heritable traits that happen without changes to the DNA sequence. The Greek prefix epi- implies features that modify the traditional genetic mechanisms of inheritance. Increasing information underscores the importance of epigenetic changes during the fetal period and infancy. The most frequently seen epigenetic changes are mediated via DNA methylation, changes in gene expression due to non-coding RNAs, and post-translational modifications of histone proteins. DNA methylation can be confirmed using methods such as bisulfite treatment, enzyme sensitivity assays, and antibody specificity-based techniques. Histone modifications are typically detected through antibody recognition. Chromatin immunoprecipitation (ChIP) is an antibody-based technology to selectively enrich specific DNA-binding proteins along with their DNA targets. Since epigenetic alterations are often reversible, modifying epigenetic marks contributing to disease development may provide an approach to designing new therapies. Gene hypermethylation and histone hypoacetylation are attractive targets for the treatment of epigenetic diseases because these epigenetic alterations are reversible. The first 1000 days of life, from conception through infancy, comprise the most-likely time-period for environmental exposures and nutrition to exert beneficial/potentially harmful epigenetic effects. During this period, a typical metabolic reprogramming induced by extrinsic factors such as allergens, viruses, pollutants, diet, or microbiome might drive cellular metabolic dysfunctions and defective immune responses in allergic diseases. Epigenetics also plays a role in the developmental origins of adult metabolic diseases.

Keywords: DNA methylation, Epigenetics, Genomic Imprinting, Histones, Infant, miRNA, Neonate, Newborn, RNA silencing.

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Keypoints

- Epigenetics is the study of heritable traits that happen without changes to the DNA sequence. Here, the Greek prefix *epi-* implies features that modify the traditional genetic mechanisms of inheritance. Increasing information underscores the importance of epigenetic changes during the fetal period and infancy.
- The most-frequently seen epigenetic changes are mediated via DNA methylation, changes in gene expression due to non-coding RNAs, and post-translational modifications of histone proteins.
- The histone tails on the nucleosome surface can undergo several enzyme-catalyzed post-translational modifications. These altered histones contain specialized structural folds in the *N*-terminal tail domain, which changes the interaction with DNA-binding proteins such as transcription factors and other binding proteins.
- Other than methylation, histones can undergo various other covalent modifications such as acetylation, ubiquitination, the addition of small ubiquitin-like modifiers (SUMO; SUMOylation), glycosylation, hydroxylation, phosphorylation, sulfation, acetylation, citrullination, crotonylation, malonylation, and ADP-ribosylation.
- The first 1000 days beginning from conception comprise the most-likely time-period for environmental exposures and nutrition to drive cellular metabolic dysfunction and defective immune responses in allergic disease. Epigenetics also play a role in the developmental origins of adult metabolic diseases.

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Introduction

In the 1940s, Conrad Waddington coined the word epigenetics, referring to indefinite genetic principles.[1–4] He described it as the causal interactions between genes and their products, thereby modulating the expression of a genotype into a particular phenotype. Nowadays, it also includes non-genetic heritable (mitotically and/or meiotically) genomic modifications involved in gene expression regulation, but not changes in DNA sequence.[5,6]

Epigenetic changes carry heritable information obtained during cell division, apart from the actual DNA sequence consisting of chemical tags that alter DNA structure and expression of genes.[7,8] These are perceived as a link with the immediate environment that enables adaptation to changing environments through extra- and intracellular cues.[9] Increasing information underscores the importance of epigenetic changes during the fetal period and infancy.

Molecular Mechanisms of Epigenetics

Most epigenetic changes occur more rapidly than genetic mutations, especially in response to environmental changes. *De novo* epigenetic mutations are seen more often than somatic DNA mutations because of the inherent, higher error rates.[10–12] Therefore, epigenetic biomarkers are mitotically and/or meiotically heritable but reversible, functional, and biologically relevant biochemical modifications of the chromatin carrying the information but not changing the nucleotide sequence of the genome.[13–17]

Table 1 lists various epigenetic mechanisms that are known to regulate gene expression. The most frequently seen epigenetic changes are mediated include DNA methylation, changes in gene expression due to non-coding RNAs, and post-translational modifications of histone proteins.[18] DNA methylation and histone modifications are the two most frequently evaluated "classical" epigenetic mechanisms.[19,20] These can alter the accessibility of genes to the transcriptional machinery and consequent regulation of gene expression, cellular hemostasis, and responses to DNA damage.[21,22] Figure 1 shows the nucleosome solenoid model. In Figure 2, euchromatin and heterochromatin are depicted.

DNA Methylation

These epigenetic changes refer to the addition of methyl groups to DNA; these frequently alter cellular reprogramming, tissue differentiation, and development.[23,24] Cytosine, and sometimes adenine, bases in DNA can undergo methylation. The 5′ methylation of cytosine in CpG dinucleotides shows as the 5-methylcytosine (5mC) bases. The CpG or CG sites are regions in DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5′ → 3′ direction. Around 60%–90% of CpGs are methylated, whereas unmethylated CpG-rich sequences are seen more frequently in the "CpG islands" located in gene promoters. The methyltransferases DNMT3a and DNMT3b carry out cytosine methylation of CpG sites, and the maintenance methyltransferase DNMT1 duplicates pre-existing methylation patterns during DNA replication.

The CpG islands (CGIs) are short-interspersed DNA sequences that are GC-rich, CpG-rich, predominantly non-methylated gene regulatory hubs. These serve as transcription initiation sites and are associated with gene promoters.[25] Transcription in more than 50% of human genes is initiated from CpG islands. Silencing of CGI promoters is achieved by CpG methylation or polycomb recruitment.[26] The CGI promoters are loaded with polymerases that create short abortive transcripts even when the associated gene is inactive.[27] This protects CGIs from the action of DNA methyltransferases, allowing these "silent" promoters to exclude DNA methylation.

Polycomb-group (PcG) genes encode multimeric chromatin proteins that bind specific histone modifications to prevent gene activation and maintain repressed chromatin domains. These multifaceted proteins were first discovered as epigenetic, global transcriptional repressors of homeotic (Hox) gene expression in Drosophila during development and differentiation.[28]

The DNA methylation of a promoter region can repress transcription by blocking transcriptional activators and through methyl CpG binding proteins (MBPs).[29] The MBPs can recognize methylated DNA and recruit co-repressors, such as HDACs, to repress gene expression.[29] The enzymes that establish, recognize, and remove DNA methylation can be categorized as writers, erasers, and readers. Writers are the enzymes that catalyze the addition of methyl groups onto cytosine residues and are comprised of Dnmts. Three members of the Dnmt family directly catalyze the addition of methyl groups onto DNA: Dnmt1, Dnmt3a, and Dnmt3b.[23] Erasers modify and remove the methyl group while readers recognize and bind to methyl groups to ultimately influence gene expression. DNA demethylation is characterized as either passive or active. Passive DNA demethylation occurs in dividing cells. Inactivation of Dnmt1 leads to unmethylation of newly incorporated cytosine, reducing the overall methylation level after each cell division, thereby causing erosion. Active DNA demethylation can occur in both dividing and

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Table 1: Mechanisms of epigenetic changes[12]

Epigenetic mechanisms	
DNA methylation	
RNA silencing/Non-coding RNAs	• Small interfering RNAs (siRNA) • MicroRNAs (miRNA)
Post-translational modifications (PTMs) of histone proteins	• Acetylation • Ubiquitination is the addition of a small ubiquitin-like modifier (SUMO; SUMOylation) • Glycosylation • Hydroxylation • Phosphorylation • Sulfation • Acetylation • Citrullination • Crotonylation • Malonylation • ADP-ribosylation
Prions	
Chromatin remodeling	
Nucleosome positioning	
Sex-specific epigenetic changes	• Genomic imprinting • Chromosome inactivation

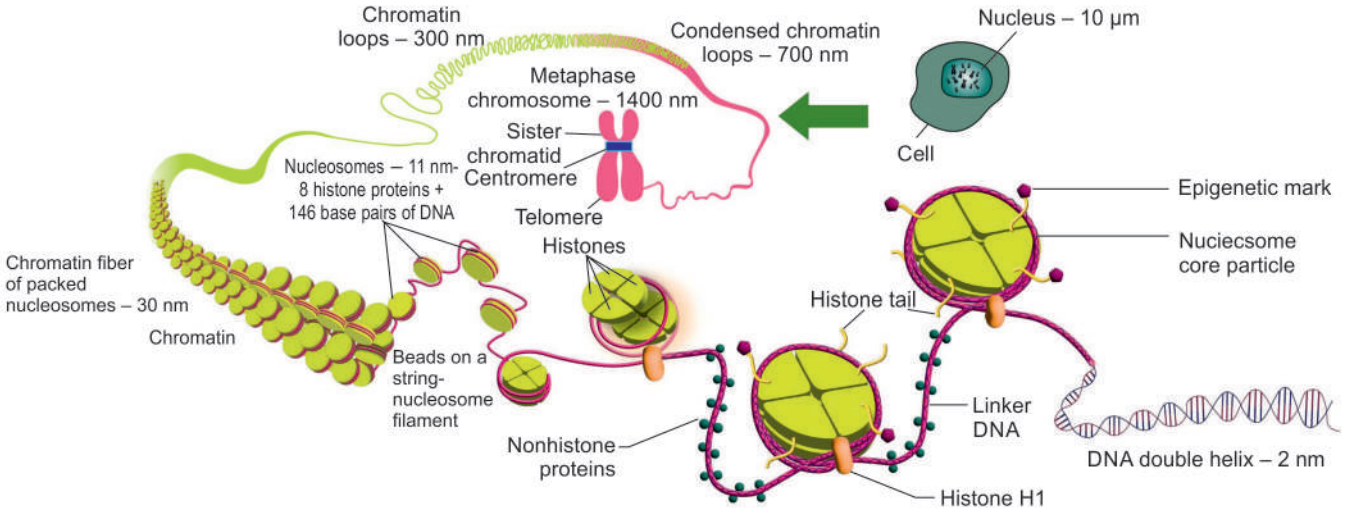


Fig. 1: Nucleosome solenoid (30 nm DNA fiber of chromatin, resulting from helical winding of ≥5 nucleosome strands). This model of chromatin organization was proposed by Kornberg and Thomas in 1974

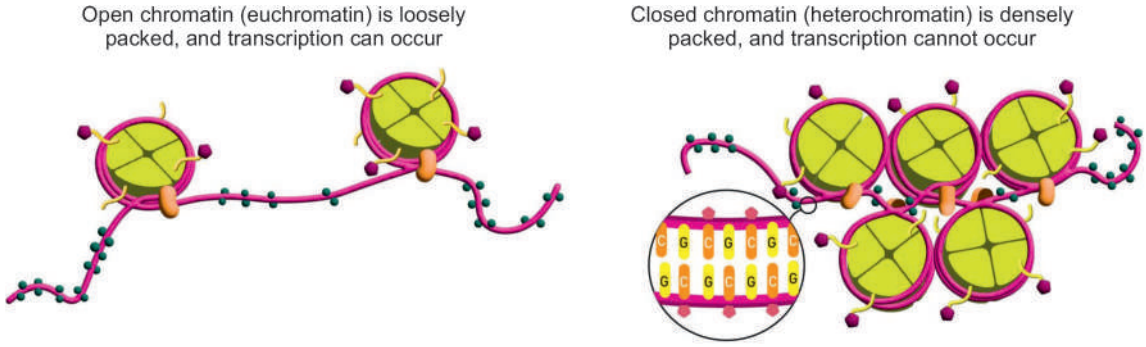


Fig. 2: Differences between euchromatin and heterochromatin

nondividing cells but requires enzymatic reactions to process the 5mC in order to revert it back to a naked cytosine. Reading DNA methylation can be done by the MBD, the UHRF, and zinc-finger proteins.[23]

Epigenetic Crosstalk

There is a crosstalk between DNA methylation and other epigenetic mechanisms, such as histone modifications and microRNA (miRNA) to regulate transcription.[23] Multiple epigenetic mechanisms interact to activate or silence gene expression and regulate transcription. Methylation is regulated by proteins such as Dnmt, and their catalytic activity is enhanced by their association with histone tails and Dnmt3L. In regions of DNA with activated transcription, Tet removes DNA methylation, and histone tails in this region often contain H3K4me3 that inhibits Dnmt binding to unmethylated CpG sites and maintains a permissive environment for transcription. Methyl binding proteins are the strongest link between DNA methylation and histone modification. Both the MBDs and the UHRF proteins interact with methylated DNA and histones to enhance gene repression.[30–32]

Non-coding RNAs

The non-coding RNAs (ncRNAs) are RNA transcripts that do not encode proteins like mRNAs but regulate post-transcriptional gene expression in physiological processes such as cellular differentiation and organ development.[33] These are epigenetic regulators of gene expression through actions on chromatin; and are categorized into (a) housekeeping or infrastructural ncRNAs that regulate cellular functions and are constitutively expressed and (b) regulatory ncRNAs which alter gene expression through complex molecular and cellular processes.[34] Infrastructural ncRNAs include ribosomal, transfer, small nuclear, and small nucleolar RNAs. Regulatory ncRNAs can be classified into microRNAs (miRNAs), Piwi-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and long non-coding RNAs (lncRNAs).[33–36] Small ncRNAs such as miRNA, siRNA, and piRNA have less than 200 nucleotides, whereas long ncRNA contains more than 200 nucleotides.[37] They can effectuate post-transcriptional silencing of gene expression by RNA processing, chromatin structure, RNA stability, chromosome segregation, transcription, and translation.[38,39] Only 3–4% of the transcripts in the genome encode for proteins, while the remaining are ncRNAs. During evolution, the spectrum of protein-coding genes has been conserved but the number of non-coding sequences has increased in proportion to the organism complexity.[40] Table 2 presents a description of the regulatory ncRNAs.

Micro (Mi)-RNAs

Micro-RNAs (Mi-RNAs) are evolutionarily conserved, small single-stranded molecules (20–24 nucleotides) that regulate the expression of around 50% of the genes in a cell at the

Table 2: Regulatory ncRNAs[35]

Type		*Length*	*Characteristic*	*Function*
miRNA	Micro RNA	20–24	miRNA produced in the nucleus as capped and polyadenylated ssRNA with an imperfectly paired stem-loop structure. Processing by Drosha and Dicer lead to the production of mature dsRNA with exact ends. The effector phase occurs primarily in the cytoplasm mediated by Ago proteins.	Perfect complementarity – Ago2-mediated cleavage of mRNA. Non-perfect complementarity – Suppression of translation or mRNA degradation (deadenylation, decapping, and exonucleolytic degradation). Transcriptional silencing and translational activation. Minor functions in transcriptional silencing and translational activation.
piRNA	PIWI-interacting RNA	24–31	Precursor ssRNA, which is modified to contain 3′-terminal 2′-O-methyl Strong preference for uridine at the 5′ end.	Silencing of transposable elements in the germline.
siRNA	Small interfering RNA	20–24	Canonical siRNAs form long, linear, perfectly base-paired dsRNA. Processed by dicer into mature siRNA with heterogeneous end-composition. Effector functions occur primarily in the cytoplasm supported by Ago proteins.	Perfect match with endonucleolytic cleavage. Non-perfect match or endonuclease-inactive RISC: Translational repression or exonucleolytic degradation Induction of heterochromatin formation Silencing of the same locus from which they are derived.
lncRNA	Long non-coding RNA	>200	Precursor of ssRNAs Many lncRNAs are subject to splicing, polyadenylation, and other post-transcriptional modifications.	Chromatin remodeling. Transcriptional regulation. Post-transcriptional regulation (splicing, TF localization). Precursors for siRNAs. Component of nuclear organelles (paraspeckles, nuclear speckles).
eRNA	Enhancer RNA	100–9000	ssRNA produced bidirectionally from enhancer regions enriched for H3K4me1, Pol II, and co-activators such as p300 Short half-life Evolutionarily conserved sequences Dynamically regulated upon signaling Expression correlates positively with nearby mRNA expression.	Mostly unknown but plays a role in transcriptional gene activation.
PAS (PASR, TSSa-RNA, tiRNA, PROMPT)	Promoter-associated RNA	16–200	Weakly expressed ssRNAs short-half life Bidirectional expression reflecting Pol II distribution.	Transcriptional regulation (interaction with Polycomb group of proteins).

PASR, promoter-associated small RNA; PROMTs, promoter upstream transcript; tiRNA, transcription initiation RNA; TSSa-RNA, transcription start site-associated RNA

post-transcriptional level.[41] These regulate immune functions and inflammatory responses. miRNAs are derived from transcripts with distinctive hairpin structures. Processing of the hairpin into the mature miRNA by Drosha and Dicer allows interaction with argonaute (Ago) proteins to form an RNA-induced silencing complex (RISC).[42–45] Strand selection for RISC depends on thermodynamic stability, with the 5′ terminus favored at the less stable end of the duplex.[41] The miRNAs then pair with mRNAs, most favorably to the 3′ untranslated region (UTR), to guide their translational repression or deadenylation and degradation. Dysregulation of miRNA expression and consequent epigenetic disruption have been correlated with altered development.[46]

Small Interfering RNA

Small interfering RNA (siRNA) is a linear, perfectly base-paired dsRNA, which is processed by Dicer into 20–24 nucleotide siRNAs that direct silencing when loaded onto RISC. The siRNAs mediate post-transcriptional silencing through RNA interference (RNAi) processes; but in contrast to miRNAs, guide strand recognition is indistinguishable.[35,38] In addition to post-transcriptional gene silencing (PTGS), siRNAs have also been found to direct sequence-specific transcriptional gene silencing by increasing epigenetic marks characteristic of heterochromatin.[38,47]

PiRNAs

Piwi (*P*-element *I*nduced *WI*mpy testis in *Drosophila*)-interacting RNA (piRNA) is the largest class of small non-coding RNA molecules expressed in animal cells. These are usually 21–35 nucleotides long, small regulatory ncRNAs. During germline development, these bind PIWI proteins to form piRNA-induced silencing complexes.[48,49] The PiRNAs can interact with mRNA with enhanced silencing of gene transcription through RISCs-mediated mRNA degradation.[35,50]

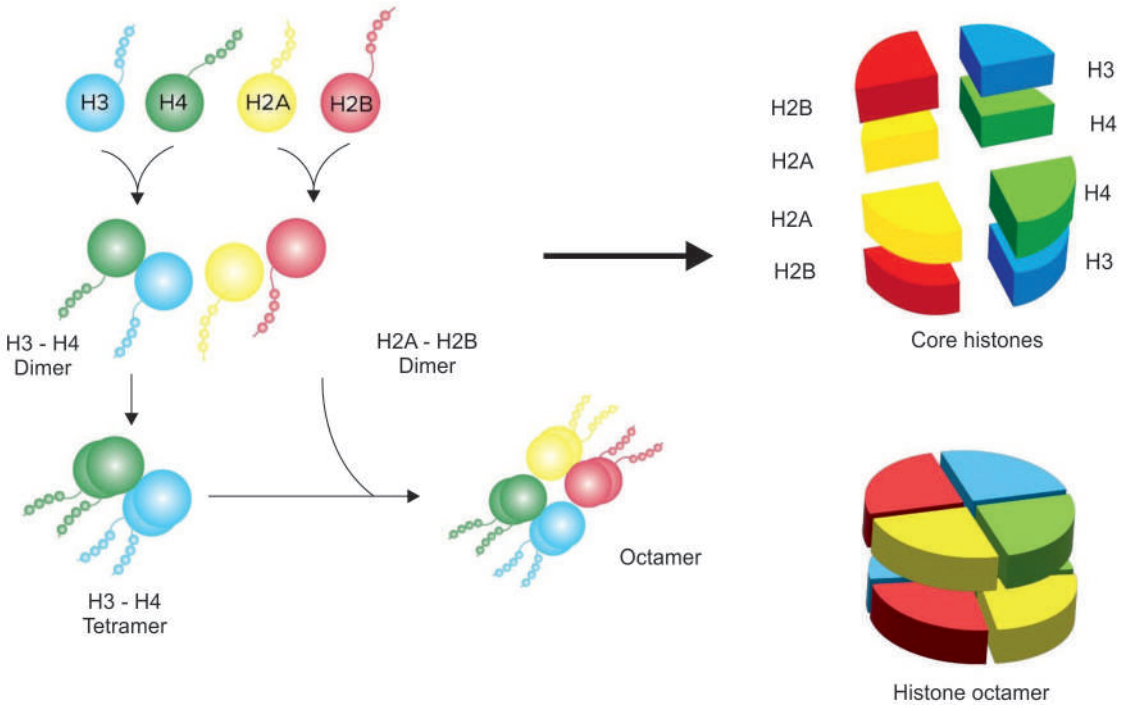


Fig. 3: Formation of histones

There is also increased suppression of transposon activity during germline development.[51] Some single-stranded precursors can mature into antisense (AS) piRNAs, which then recognize and target the cleavage of transposons by associated PIWI-proteins. This sequence generates additional sense-piRNAs from the target transposon sequence, mostly in germline and sometimes in somatic cells.[52,53] The PiRNAs may also have a role in the regulation of the cell cycle of mesenchymal stem cells.[54]

Long Non-coding (Lnc)-RNAs

Long non-coding RNAs (Lnc-RNAs) comprise the largest subgroup of non-protein-coding transcripts. These are typically more than 200 nucleotides in length. Based on the proximity to protein-coding genes, lnc-RNAs are usually classified into five categories: sense, AS, bidirectional, intronic, and intergenic.[36] A subgroup of lncRNAs, named large intergenic non-coding RNAs (lincRNAs), has been described based on a distinctive chromatin signature that marks actively transcribed genes.[55,56] These are transcribed by RNA polymerase II. There is considerable mechanistic diversity due to variations in interaction with RNA binding proteins (RBPs) at specific DNA regions.[57] Due to the presence of a poly(A) tail, these can be detected by qRT-PCR through a poly-A tailing method.

Table 2 summarizes the role of regulatory non-coding RNAs.

Post-translational Modifications (PTMs) of Histone Proteins

The histone tails on the nucleosome surface can undergo several enzyme-catalyzed PTMs.[58,59] These modifications can alter the net charge, inter-nucleosomal interactions, and the chromatin structure.[60,61] These altered histones contain specialized structural folds in the *N*-terminal tail domain, which changes the interaction with DNA-binding proteins such as transcription factors and other binding proteins. Figure 3 explains the formation of histones. In Fig. 4, we present a detailed structure of histones. Histone–DNA and histone–histone interactions occur in the globular domain.

Covalent modifications include acetylation, ubiquitination, the addition of small ubiquitin-like modifier (SUMO; SUMOylation), glycosylation, hydroxylation, phosphorylation, sulfation, acetylation, citrullination, crotonylation, malonylation, and ADP-ribosylation.[61] These affect gene expression by altering interactions of the positively charged *N*-termini of histones with negatively charged DNA and creating binding sites for modified histone residues.[62]

Histone Acetylation

Histone acetylation occurs at conserved lysine residues on the *N*-terminal tails.[58] Most underlying modifications are seen in H3 and H4, not in H2A and H2B. Key positions for acetylation are lysine K9 and K14 on histone H3; and K5, K8, K12, and K16 on histone H4.[63,64] The degree of histone acetylation is directly proportional to transcriptional activity. The acetylation and deacetylation of histones are a dynamic process managed by two enzyme systems, the histone acetyltransferases (HATs) and histone deacetylases (HDACs).[63]

The HATs promote the transfer of acetyl moieties from acetyl coenzyme A to lysine residues of histone proteins. The HATs and HDACs are two counteracting enzyme families that control the acetylation state of protein lysine residues in the *N*-terminal extensions of the core histones. The activity of HDACs is controlled by targeted recruitment, protein–protein interactions, and post-translational modifications. The HDACs control cell cycle progression, survival, and differentiation; these enzymes were first noted in malignant transformation. Therefore, HDAC inhibitors were developed as antineoplastic drugs.

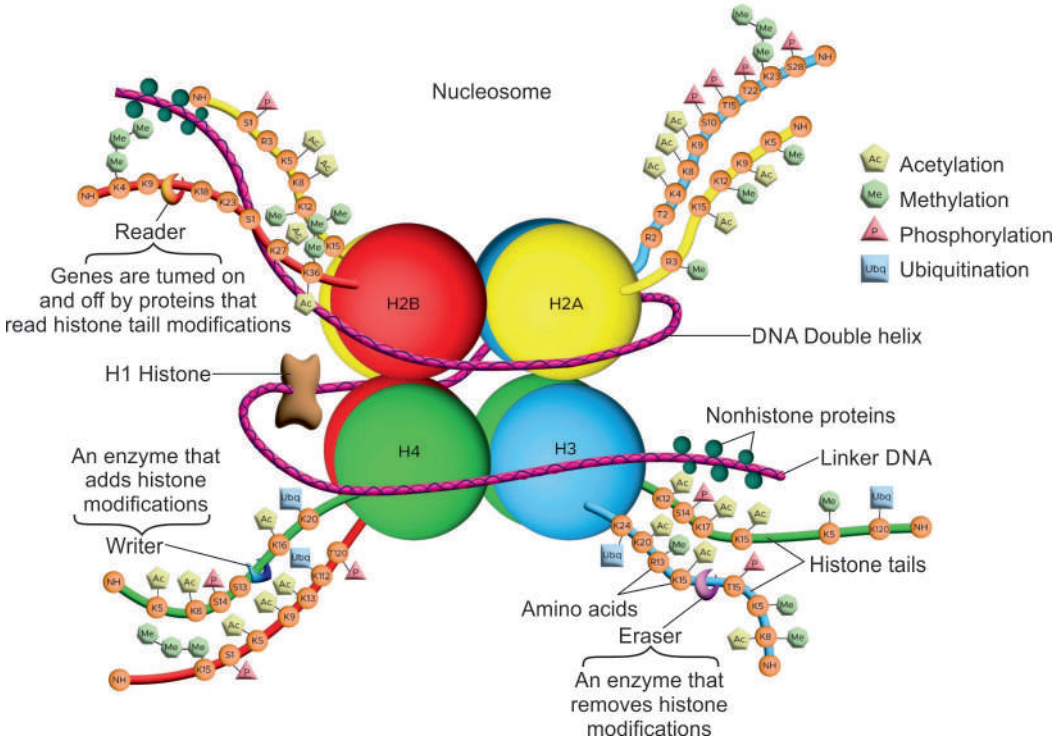


Fig. 4: Detailed structure of histones

Table 3: Classification of histone deacetylase inhibitors

Class	*Compound*
Hydroxamate	Trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), m-carboxycinnamic acid bishydroxamide (CBHA), LAQ-824
Cyclic peptide	Depsipeptide, apicidin, and the CHAPs
Aliphatic acid	Valproic acid (VA), phenylbutyrate (PB)
Benzamide	MS-275, CI-994
Electrophilic ketone	Trifluoromethyl ketones, alpha-ketoamides.

The HDACs suppress transcription; inhibiting these enzymes promotes histone hyperacetylation transcriptional activation of some genes.[63] Several histone deacetylase inhibitors (HDACIs), small specific/global inhibitors of HDACs with distinct structural characteristics, are known: hydroxamates, cyclic peptides, aliphatic acids, benzamides, and electrophilic ketones (Table 3).[65]

Histone Methylation

Histone methylation, an irreversible epigenetic mark, can both activate and repress gene expression.[66] Methylation of H3/K9 and H4/K20 are associated with heterochromatin formation; H3/K9 and K27 with transcriptional silencing; and K4, K36, and K79 of histone H3 associated with gene activity.[66,67] Methylation of histones and DNA can occur together during chromatin remodeling.[68,69]

Prions

Prions are infectious conformational states of some proteins, which transfer their misfolded state to other proteins, creating new, diverse heritable traits without any changes in the nucleic acid sequence.[70] These may be involved in the pathogenicity of some neurodegenerative diseases.

Chromatin Remodeling

Altered spatial organization and chromatin interactions can contribute to some human diseases, such as Rubinstein–Taybi syndrome (RSTS).[70] These conditions are being recognized as Mendelian disorders of the epigenetic machinery and are being classified as "chromatinopathies."[71] The RSTS is caused by *de novo* mutations in epigenetics-associated genes such as the cAMP response element-binding protein (CREBBP). This protein encoded by this gene is referred to as CBP. The EP300 gene encodes the p300 protein, a CBP homolog. The CBP has an intrinsic histone acetyltransferase activity required for CREB-mediated gene expression. Protein acetyltransferase activity in CBP destabilizes promoter-bound nucleosomes, leading to transcriptional activation. Overall, chromatin structure is dynamic and precisely controls cellular processes such as gene expression.[72] Global chromatin remodeling plays a major part in gene expression and epigenetic memory.[73]

Chromatin acts as a dynamic signal platform through active histone modifications. Chromatin structure is modified either by breaking the interactions between nucleosomes or by recruitment of protein factors to the unraveled nucleosomes.[74] Histone-modifying enzymes include acetyltransferases, methyltransferases, serine/threonine kinases, ubiquitin ligases, and proline isomerases. The encoded covalent modifications affect the physical remodeling of chromatin structure or recruitment of signaling complexes that drive/repress transcription.[75] Histone modifications and chromatin remodeling are critical for gene expression during the memory processes and learning.

Nucleosome Positioning

Nucleosomes contain a histone core inside a DNA bundle (Fig. 1). The position and modifications of nucleosomes are key to altered genome regulation in developmental defects and cancer.[76]

Nucleosome-remodeling ATPases maintain the chromatin in a dynamic state responsive to environmental, metabolic, and developmental cues. These enzymes undergo conformational changes that promote binding to and hydrolysis of ATP and interaction with DNA and histones. The resulting histone–DNA interactions in target nucleosomes may lead to the complete or partial disassembly of nucleosomes, the exchange of histones for variants, the assembly of nucleosomes, and/or the movement of histone octamers on DNA. Remodeling can maintain DNA sequences close to interacting proteins. It can also promote histone modifications or RNA metabolism, which is important for stable epigenetics.[77]

In chromatin, the DNA sequence is occluded by histones and non-histone chromatin components. Access to DNA is restricted due to the compact chromatin organization. The proteins cannot easily associate with DNA strands on the nucleosomal histone surface. Also, the nucleosomal DNA shows major tertiary structural curves around the histone octamer. There are also post-translational modifications in histones that further fold the nucleosomal fibers into "higher order" structures which are even less accessible. Nucleosome "remodeling" enzymes allow DNA access to be regulated by changing histone–DNA interactions as a means of disrupting, assembling, or moving nucleosomes and liberating segments of DNA by complete or partial disassembly, alteration of the composition of nucleosomes and by affecting the folding of nucleosomal fibers.[78–82]

Generally, nucleosomes are stable structures due to the cumulative effect of many weak histone–DNA interactions. Remodeling reactions require biochemical coupling in histones to ATP hydrolysis and are reversible. These include disruption of histone–DNA interactions, the sliding of a histone octamer on or off a particular DNA sequence, or alteration of the composition of histone variants. Remodeling factors are multisubunit complexes; these enzymes, despite the dynamic nature of the chromatin transitions, are involved in the assembly and propagation of stable epigenetic states. The typical roles for nucleosome remodeling include regulatory elements that affect expression programs of specific genes, nucleosome assembly, and exchange of histone variants. Serial deletions that alter the 3-dimensional interactions between heterochromatic nucleosome depleted regions (NDRs) have helped understand the relationship between inter-NDR distance and defects in nucleosome positioning. Poor nucleosome positioning can also alter heterochromatin stability.[83]

Sex-specific Epigenetic Mechanisms

Genomic Imprinting

Genomic imprinting involves mono-allelic expression depending on the parental origin; the gamete-specific epigenetic modifications result in differential expression of the two parental alleles in somatic cells. DNA gets tagged in a sex-dependent manner, resulting in differential gene expression in accordance with the parent of origin. Imprinting occurs during gametogenesis and is maintained by DNA replication in the somatic cells.

Imprinting control regions (ICRs) are composed of repetitive DNA sequences nearby or in imprinted genes; removal of an ICR results in loss of imprinting. Epigenetic modifiers of gene expression, such as DNA methylation, histone modifications, non-RNA factors, and higher-order chromatin formations within ICRs can help maintain the imprinted state. The ICRs can be important components of nucleation sites for gene silencing/activation. These are also associated with enhancers and boundary elements to restrict imprinted regulation to specific domains.

Most imprinted genes share common ICRs in clusters that direct parent-specific regulation of multiple genes. The ICRs contain differentially methylated regions (DMRs) that achieve parent-specific DNA methylation tags either in the germline or in somatic cells. Histone acetylation creates an accessible chromatin conformation. In contrast, histone deacetylation can initiate histone methylation. Altered chromatin conformation can promote silencing and the formation of heterochromatin.[84] Histone methylation can activate/repress transcription depending upon methylation of specific lysine: histone 3-lysine 9 (H3K9), histone 4-lysine 20 (H4K20), and histone 3-lysine 27 (H3K27) are silencing modifications, whereas histone 3-lysine 4 (H3K4) methylation typically activates chromatin. Histone modifications in imprinted regions can promote the formation of higher-order chromatin. Transcriptional inactivation of an imprinted allele involves the formation of heterochromatin.

RNA interference is a post-transcriptional silencing mechanism in which double-stranded RNA (dsRNA) promotes the formation of complementary RNA transcripts by forming RNA silencing complexes (RISCs). The RNA-I silencing pathway helps recruit DNA methyltransferases and other factors to promote higher-order chromatin structures.[85] Non-coding RNA and RNA-I are also regulators of genomic imprinting. This might involve histone acetylation/methylation and/or DNA methylation. Differential allele-specific DNA methylation is localized to regions termed differentially methylated regions. Differential methylation of parental alleles can be involved in this process. These domains often contain many imprinted genes, such as *Igf2/H19*, *Cdkn1c/Kcnq1*, and *Zac*, that play important roles in growth and postnatal development. Many imprinting gene loci are related to neonatal diseases.

The insulin-like growth factor (IGF) system, including IGF-1 and IGF-2, is one of the most important endocrine and paracrine growth factor systems regulating fetal and placental growth. The IGF-2 and H19 genes are important imprinted genes.[86,87] The IGF-2 assays are standard for determining the presence of genomic imprinting.[88] The H19 is maternally and IGF2 is paternally expressed.[89,90] The H19 has one ICR located upstream of the H19 gene and is also paternally methylated.[91–93] Once established, paternal-specific methylation is identified and maintained in the somatic cells.[94,95]

Diagnostic Methods

Different methods have been developed to identify and assess the scale of epigenetic alterations, focusing on DNA methylation and histone modification detection. Bisulfite, enzyme sensitivity, and antibody specificity-based techniques are used for DNA methylation, whereas histone modifications are detected by antibody recognition.[96] To detect DNA methylation, traditional techniques such as polymerase chain reactions (PCRs) or cloning methods are not applicable because methyl groups are not copied during PCR amplification.[97] Pretreatment methods can be used to study the original methylated DNA strands to discriminate methylation from non-methylation regions.[98,99]

Chromatin immunoprecipitation (ChIP) is a reliable way of detecting histone modifications and altered chromatin structures.[100] The ChIP utilizes specific antibodies to recognize specific histone modifications and/or epigenetic modulators along with particular DNA fragments, which allows for assigning

Table 4: Laboratory methods to decipher epigenetic signatures[96]

Technique	*Types*	*Description*
Restriction digestion-based techniques	MS-AFLP	Methylation-sensitive amplitude fragment length polymorphism
	DMH	Differential methylation hybridization
	CHARM	Comprehensive-high throughput arrays for relative methylation
	MMASS	Microarray-based methylation assessment of single samples
	HELP	HpaII tiny fragment enrichment by ligation-mediated PCR
	MS-MLPA	Methylation-specific multiplex ligation-dependent probe amplification
	LUMA	Luminometric methylation assay
	RLGS	Restriction landmark genomic scanning
	MCA	Methylated CpG island amplification
Bisulfite treatment-based techniques	MSP	Methylation-specific PCR
	MSP-ISH	Methylation-specific PCR *in situ* hybridization
	BSP	Bisulfite sequencing PCR
	MS-RTPCR	Methylation-sensitive real-time PCR
	HRM	High-resolution melting
	Bisulfited DNA pyrosequencing	
	SNuPE	Methylation-sensitive single nucleotide primer extension
	MALDI-TOF	Matrix-assisted laser desorption ionization time-of-flight
	COBRA	Combined bisulfite restriction analysis
Affinity-based technology	MeDIP	Methylated DNA immunoprecipitation
	MBD protein affinity approach	Methyl binding domain protein affinity approach
	5hmC detection	5-Hydroxymethylcytosine detection
Histone modification analysis	ChIP assay	Chromatin immunoprecipitation assay
	Modified-ChIP-based methods	Carrier ChIP (CChIP), quick and quantitative ChIP (Q2 ChIP), MicroChIP (μChIP), Fast ChIP, and Matrix ChIP.
	Site-specific analysis of histone modifications	
	DNAse I hypersensitivity analysis of chromatin structure	

locus-specific functions of histone modifications or transcriptional factor complexes.[101] Sequencing-based ChIP methods such as ChIP-chip or ChIP-seq can analyze protein–DNA binding events and histone modifications at different loci for non-specific modification patterns.[102,103] Table 4 provides an overview of these techniques for detecting epigenetic effects.

Uses in Neonates

Environmental factors typically induce epigenetic effects during the first 1000 days after conception. Extrinsic factors such as allergens, viruses, pollutants, diet, and/or microbiome can activate atypical metabolic reprogramming, metabolic dysfunctions, and defective immune responses, such as in allergic disease.[13] Flowchart 1 summarizes the major epigenetic exposures in the perinatal period.

miRNAs in Neonatal Sepsis

During infections, epigenetic changes may occur, leading to reprogramming of gene expression. Post-transcriptional regulation by short non-coding RNAs (microRNAs) may have a role in the pathophysiology of neonatal sepsis and may be used as potential biomarkers.[104] MicroRNAs of 22-nucleotide length regulate immune functions and inflammatory response.

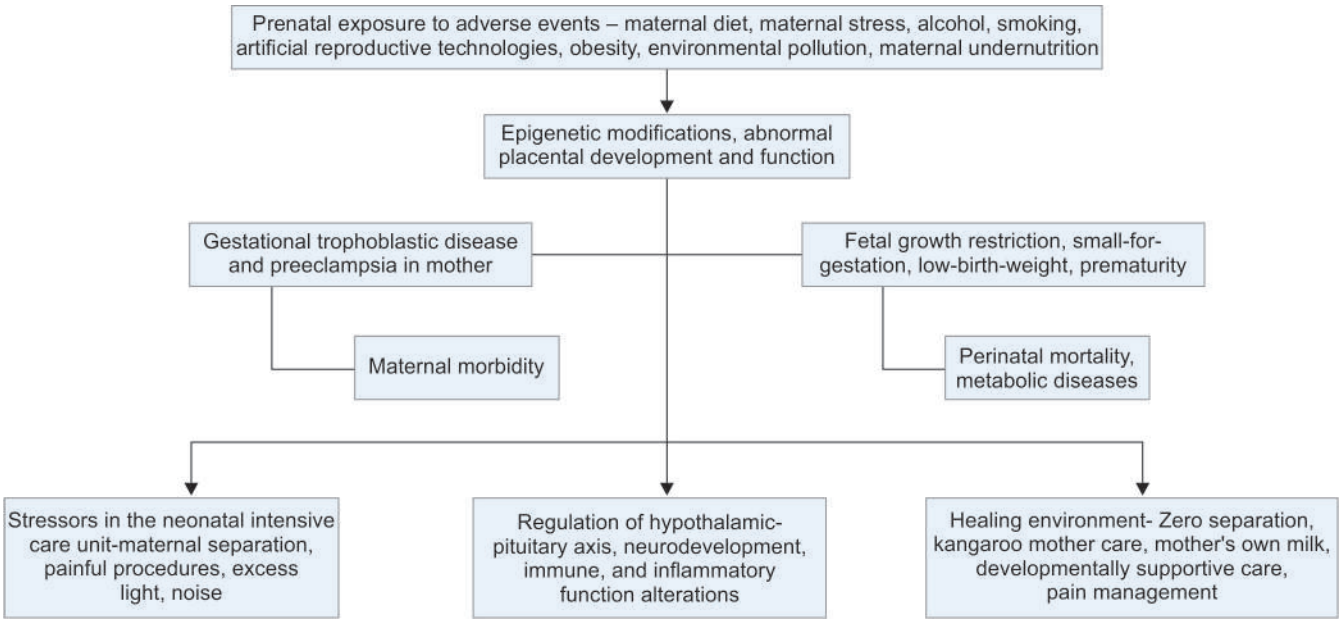
Necrotizing Enterocolitis (NEC)

In neonatal pathologies, epigenetic changes are used to identify targets for therapeutic intervention and predict outcomes.[12]

Persistent Pulmonary Hypertension of the Newborn

Epigenetics regulates this endothelial cell-specific expression of eNOS, including DNA methylation, histone acetylation and methylation, and RNA interference.[58] The PPHN significantly increases DNA CpG methylation in the eNOS promoter and decreases ten eleven translocase demethylases (TET) activity in pulmonary artery endothelial cells (PAEC). The PPHN decreased Sp1 occupancy and density of the active mark, lysine 12 acetylation of histone 4, and increased density of the repression mark, lysine 9 trimethylation of histone 3 around Sp1 binding sites in endothelial nitric oxide synthase (eNOS) promoter. These results suggest that epigenetic modifications are primed to decrease Sp1-binding at the eNOS gene promoter in PPHN. Reduced expression of eNOS, a key mediator of perinatal transition, characterizes PPHN.

Flowchart 1: Epigenetic exposures in the perinatal period



Congenital Disorders

Epigenetic modifications have a role in congenital disorders (such as Silver–Russell and Beckwith–Wiedemann syndrome), transient neonatal diabetes mellitus (TNDM), intrauterine growth retardation (IUGR), and PPHN.[58] Epigenetics is also involved in genomic imprinting and X-chromosome inactivation in humans, and the failure of this mechanism causes a subset of congenital syndromes.[105] The SNRPN gene, located in chromosome 15q12, is an imprinted gene where the maternal allele is inactivated and the paternal allele is expressed. The UBE3A gene is another imprinted gene where the paternal allele is inactivated, and the maternal allele is expressed. Failure to express the SNRPN gene due to deletion of paternal chromosome 15 or abnormal methylation of the paternal allele results in Prader–Willi syndrome. Failure of expression of the UBE3A gene due to either deletion of maternal chromosome 15 or abnormal methylation of the maternal allele results in Angelman syndrome.[106,107] Around 100 imprinted genes clustered in certain genome regions have been identified. In genomic imprinting, the inactivated allele is methylated in its promoter region, whereas the expressing allele is not methylated; whereas in some disorders, both alleles are methylated, leading to a complete lack of gene expression.

Lyonization of the X-chromosome occurs in females, but in some rare diseases, both X-chromosomes are active, leading to severe developmental delay. DNA is methylated by specific enzymes, such as DNA methyltransferases (DNMTs). In a disease with immunodeficiency [immunodeficiency, centromere instability (ICF) syndrome], mutant DNMT3B fails to methylate DNA in certain genomic regions. Genes are suppressed by epigenetic mechanisms through proteins, such as the methyl CpG- binding proteins. In Rett syndrome, a mutant methyl-CpG binding protein 2 (MeCP2) fails to repress its target genes.

Epigenetics and Neonatal Nutrition

Epigenetic changes have long-lasting effects on gene expression and are induced by the early development environment. Epigenetic DNA imprinting activity is the most active from preconception to early infancy and modifies the risk for non-communicable disorders later in life, such as cardiovascular diseases, metabolic diseases, and diseases of the reproductive system.[108]

Genetics, Epigenetics, and Transcriptomics of Preterm Birth

Maternal toxic exposure to heavy metals, air pollution, and pesticides has been correlated with reduced placental methylation, which may cause genomic instability and mutations.[109] Epigenome-wide association meta-analysis studies (EWAS) have shown that many prenatal exposures associated with spontaneous preterm birth also change DNA methylation in cord blood. The EWAS has shown reproducible associations between blood DNA methylation in newborns and maternal folate levels, exposure to smoking during pregnancy, air pollutants, and exposure to heavy metals.[110–113]

Premature uterine contractions have been associated with pathogenic variants of the sarcomere gene TTN and transcriptomic variations of sarcomeric premature uterine contraction genes regulated by epigenetic factors, including methylation and long non-coding RNAs.[114] Maternal age is an independent risk factor for preterm birth. Inter-individual differences between chronological and biological age have also been noted, which might be related to genetic background and environmental exposures. Estimation of biological age using genome-wide DNA methylation has found an association between a mother's biological age and gestational age at delivery.[115]

Epigenetics and the Placenta

Placental growth and function, pregnancy maintenance, and parturition are under epigenetic control.[116] Prostaglandin biosynthesis enzyme (prostaglandin H synthase-2) can be modified by alteration of histone acetylation status and DNA methylation status in the human placenta.[117] These inflammatory mediators affect uteroplacental hemodynamics and are critical in the

mechanisms of labor. The regulatory mechanisms extend back in pregnancy till implantation.[118]

Candidate Genes

Many epigenetically modifiable genes may promote programmed changes in growth and insulin sensitivity in small-for-date and/or premature infants. Changes in methylation at the imprinted IGF-2 receptor gene could lead to decreased IGF-II activity at birth.[119,120] Overexpression of RASGRF1, which regulates postnatal growth and is known to be imprinted in rodents, could explain the taller stature and higher IGF-I levels in IVF children. Under-expression of RASGRF1, which is known to be imprinted in rodents, could explain poor growth in premature and small-for-date infants.[121] There appears to be a critical window in early life in which under-nutrition might cause aberrant methylation of LEP and in turn, persistent insulin resistance with later obesity and hypertension. The GRB10 is another imprinted gene that acts to inhibit insulin and IGF-I receptor signaling and its hypomethylation during the fetal or early neonatal periods can lead to insulin resistance, poor growth, and abnormalities in the GH/IGF-I axis as observed in SGA and premature children.[116,122]

Possible Therapeutic Implications

Since epigenetic alterations are reversible, modifying epigenetic marks contributes to disease development may provide an approach to designing new therapies.[65] Gene hypermethylation and histone hypoacetylation are attractive targets for treating epigenetic diseases because these changes could be reversible. Despite limitations such as non-specific activation of genes and transposable elements in normal cells, corrected epigenetic modifications may revert to their previous state because of the reversible nature of DNA methylation and histone modification patterns. However, this may be prevented with continued treatment.

Epigenetic dysregulation leads to the initiation and progression of some cancers. Global DNA hypomethylation has been noted in altered growth. Promoter CpG hypomethylation can promote gene expression. Promoter CpG hypomethylation can increase gene expression. DNA methylation refers to the post-synthetic methylation of cytosine bases at position 5 of the pyrimidine ring by a DNA methyltransferase (DNMT), which catalyzes the transfer of the methyl group from S-adenosylmethionine (SAM) to cytosine to form 5-methylcytosine and S-adenosylhomocysteine (SAH). Diets deficient in methyl donor precursors (folate, methionine, and choline) may play a role in DNA hypomethylation. The DNA hypomethylation has been detected in those receiving low dietary folate and can be reversed by folate repletion. Histone acetylation and deacetylation status may be altered by inhibiting HATs or HDACs. Gene silencing is associated with histone deacetylation, which is catalyzed in human cells by at least three classes of HDACs (class I, II, and III).

Table 5 summarizes the definitions associated with epigenetics.

Table 5: Definitions associated with epigenetics[123]

Epigenetics	Non-genetic heritable (mitotically and/or meiotically) genomic modifications involved in gene expression regulation but do not entail a change in DNA sequence.[5,6]
Gene	The fundamental physical and functional unit of heredity, which carries information from one generation to the next.
Chromosome	A linear end-to-end arrangement of genes and other DNA, sometimes with associated protein and RNA.
Chromatin	Chromatin refers to a mixture of DNA and proteins that form the chromosomes.[124,125] Histones package the massive amount of DNA in a genome into a highly compact form that can fit in the cell nucleus.
Euchromatin	Euchromatin is defined as the area of the chromosome which is rich in genes that actively participate in the transcription process.[126]
Heterochromatin	Heterochromatin is a tightly packed form of DNA in the nucleus that is so compactly organized that it is inaccessible to the proteins involved in gene expression. Heterochromatin is highly condensed, gene-poor, and transcriptionally silent, whereas euchromatin is less condensed, gene-rich, and more accessible to transcription (Fig. 2).[126]
Nucleosome	A nucleosome is the basic repeating subunit of chromatin. It has a nucleosome 'core' linker DNA, and a linker histone.[127] It comprises a segment of DNA wrapped around eight histone proteins, known as histone octamer. The octamer is made up of two copies of each of the histone proteins: H2A, H2B, H3, and H4.
Histone	Histones are basic proteins found in chromosomes that help pack and organize DNA helix in chromatin fiber in the nucleus (Fig. 4).
Transcription	The synthesis of RNA from a DNA template.
Methylation	The process by which methyl groups are added to the DNA, and contributes to cellular reprogramming, tissue differentiation, and normal development.[23,24]
CpG island	CpG islands (CGIs) are short, interspersed DNA sequences that deviate significantly from the average genomic pattern by being GC-rich, CpG-rich, and predominantly non-methylated and are the sites of transcription initiation.[26]
Polycomb proteins	*Polycomb*-group (PcG) genes encode chromatin proteins involved in stable and heritable transcriptional silencing. PcG proteins participate in distinct multimeric complexes that deposit or bind to specific histone modifications (e.g., H3K27me3 and H2AK119ub1) to prevent gene activation and maintain repressed chromatin domains. PcG proteins can silence gene expression globally, particularly during development and differentiation.[28]
RNA silencing	RNA silencing or RNA interference is an evolutionarily conserved gene inactivation system by which gene expression is negatively regulated by non-coding RNAs such as microRNAs.
small interfering RNAs (siRNA)	A class of double-stranded RNA, 20–24 base pairs in length, which interferes with the expression of specific genes with complementary nucleotide sequences by degrading mRNA after transcription, preventing translation.[128]

(Contd...)

Table 5: *(Contd...)*

microRNA s (miRNA)	MicroRNAs are small, single-stranded, non-coding RNA molecules containing 21–23 nucleotides involved in RNA silencing and post-transcriptional regulation of gene expression.[129]
piRNA	piRNAs are a group of small ncRNAs with approximately 21–35 nucleotides in length. The piRNA is known to associate with PIWI proteins and form piRNA-induced silencing complexes during germline development.[49]
lncRNA	Long non-coding RNAs are RNA transcripts of more than 200 nucleotides that are not translated into protein. They are involved in epigenetic regulation, chromatin remodeling, and protein metabolism control. The term 'lncRNAs' encompasses RNA polymerase I (Pol I), Pol II, and Pol III transcribed RNAs, and RNAs from processed introns.[130]
Genomic imprinting	It is the process by which only one copy of a gene (maternal or paternal) in an individual is expressed while the other copy is suppressed.[131]
ChIP technique	An antibody-based technology that selectively enriches specific DNA-binding proteins and their DNA targets.[132]
Developmental Origins of Adult Disease Hypothesis or Barker's theory	According to the DOHaD hypothesis, epigenetic adaptations are made to the fetal/neonatal DNA in response to environmental influences.[1] The hypothesis further explains that the body responds to the environment and makes genetic changes (predictive adaptations) in anticipation of a presumed future environment. If the predictive adaptations are incorrect, then the metabolic state of the individual is altered to a degree whereby the risk of chronic disease in adulthood is increased; research also shows that the increased risk might be transgenerational.[133]
EWAS	Epigenome-wide association studies (EWASes) investigate the association between a phenotype and epigenetic variants, most commonly DNA methylation.[134,135]

Future Directions

Epigenetic alterations are crucial in the first 1000 days of life. Modifying epigenetic marks can play a role in developing new therapies. There is a need to unravel the complete repertoire of mechanisms of epigenetic modifications. Recent research is also delving into epigenetics as a causative mechanism in the pathogenesis of many neonatal disorders. Epigenetic remodeling of the genome plays a role in the phenotypic expression of various health and disease states in the newborn. Future efforts should be directed toward the possible therapeutic implications of epigenetics.

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CASE REPORT

Not Every Massive Cardiomegaly in a Newborn Infant is due to an Ebstein's Anomaly or a Large Pericardial Effusion

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Abstract

In neonates, massive cardiomegaly on chest X-rays is an infrequent but concerning finding. These observations are ascribed most frequently to tricuspid valve malformations as in Ebstein's anomaly and to large pericardial effusions. We recently treated a 40 weeks/3 kg male infant born to a 23-year-old primigravida mother after an uneventful, carefully followed pregnancy. The infant developed respiratory distress soon after birth, and a massively enlarged cardiac silhouette was noted on initial evaluation. We investigated the aforementioned possibilities of Ebstein's anomaly or a massive pericardial effusion, but this infant turned out to have a large intrathoracic cystic mass in the left hemithorax. The differential diagnosis was a bronchogenic vs gastrointestinal duplication cyst. The mediastinum was displaced towards the right side. The heart and major vessels were all normal in size. On postnatal day 8, a left posterolateral thoracotomy was performed, and a giant cystic tumor was dissected. There was a tense capsule attached to the lower lobe of the left lung, posterior pleura, esophagus, descending aortic artery, and diaphragm. The surrounding lung tissue was largely intact. Histopathology of the cyst wall showed features of both gastric and small intestinal mucosa, which was consistent with the findings seen in a broad group of anomalies known as bronchopulmonary foregut malformations. We need to consider a wider list of entities in the differential diagnosis of a massively enlarged cardiac silhouette in an infant with respiratory distress.

Keywords: Bronchogenic cyst, Bronchogenic cyst, Case report, Cerebral/hepatic arterio-venous malformations, Fetal cardiomyopathy, Gastrointestinal duplication cyst, Gastrointestinal duplication cyst, Intrathoracic cyst, Neonate, Respiratory distress.

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Key Points

- In neonates, detection of a massive cardiomegaly on chest X-rays is an infrequent but concerning finding. These observations are ascribed most frequently to tricuspid valve malformations such as in Ebstein's anomaly or to large pericardial effusions.
- We recently treated a full-term infant with significant cardiomegaly. Further investigations showed a large cyst in the left hemithorax. The heart and major vessels all turned out to be normal. The differential diagnosis for this cystic mass was a bronchogenic vs gastrointestinal duplication cyst.
- Histopathology of the cyst wall showed features of both gastric and small intestinal mucosa, which was consistent with the findings seen in the so-called bronchopulmonary foregut malformations.
- We need to consider a wider list of entities in the differential diagnosis of a massively enlarged cardiac silhouette in an infant with respiratory distress.

Introduction

In neonates, a massive "wall-to-wall" cardiomegaly on chest X-rays (CXRs) is an infrequent but concerning finding.[1–4] These observations are ascribed most frequently to tricuspid valve malformations as in Ebstein's anomaly and to large pericardial effusions.[1–8] In Ebstein's anomaly, the right atrium becomes massively dilated with atrialization of the right ventricle.[9] In some other cases, large pericardial effusions can enlarge the cardiac silhouette.[10,11] A few cases with radiographically notable cardiomegaly turned out to be due to fetal cardiomyopathy and cerebral/hepatic arterio-venous malformations.[8,12,13] Large cardiomegaly in neonates can be an important indicator of an underlying cardiac or systemic disease and hence calls for expedient evaluation.[8,12,14]

We recently treated a 40 weeks/3 kg male infant born to a 23-year-old G_1P_0 mother after an uneventful, carefully-followed pregnancy. In the third trimester, the obstetric ultrasound study showed dextrocardia with a lesion in the left lung that was suggestive of type I cystic adenomatoid malformation.[15,16] However, the pediatric team did not have this information at the time of delivery. The infant was delivered by a scheduled cesarean section; no resuscitation was needed at birth, and the Apgar scores were 7 and 8 at 1 and 5 min, respectively. However, he developed respiratory distress soon after birth with tachypnea, nasal flaring, and intermittent expiratory moaning. He was provided supplemental

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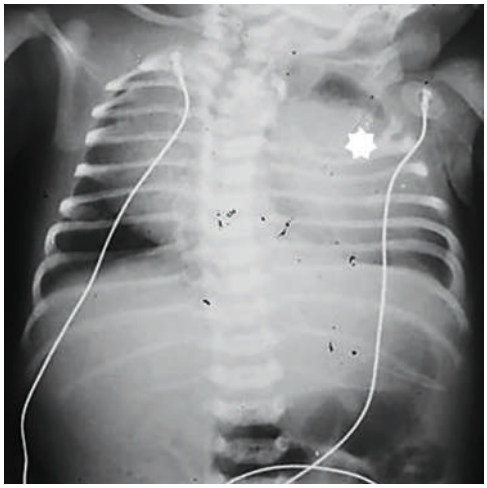


Fig. 1: Anteroposterior chest x-ray with enlarged cardiac silhouette, suggesting massive "wall-to-wall" cardiomegaly. T2 and T3 vertebral bodies show abnormalities

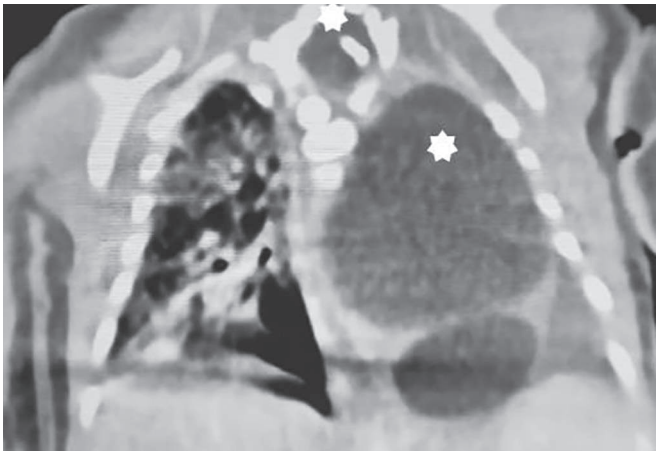


Fig. 2: Contrast-enhanced chest CT scan showed a voluminous cystic tumor in the left pleural space containing liquid density with a significant mass effect on the left lung as well as the mediastinum. In the bone window, vertebral and costal fusion anomalies were observed affecting the T2 and T3 vertebral bodies

oxygen using a headbox with a flow of 5 liters/min.[17] The laboratory profile, including complete blood counts, blood gases, electrolytes and renal profile, thyroid profile, coagulation profile, and tests for perinatal TORCH (toxoplasmosis, rubella cytomegalovirus, herpes simplex, and human immunodeficiency virus) infections, was all within normal limits.[18] The CXR showed a massive cardiac silhouette, suggesting major cardiomegaly (Fig. 1).[19,20]

Neonatal echocardiography was difficult to obtain in our unit during the night, and so we obtained magnetic resonance (MR) images of the chest. A large cyst was noted in the left hemithorax; the differential diagnosis included bronchogenic and gastrointestinal duplication cysts. The mediastinum was displaced towards the right side. The heart was normal in size, the ascending aorta measured 2.7 mm in diameter, and the pulmonary trunks were 9 mm in transverse diameter. The superior and inferior vena cavae were normally located without any signs of occlusion. A pleural effusion with a maximum thickness of 19 mm was seen. MR angiography was normal. The left lower pulmonary lobe showed areas of atelectasis, so a computed tomography (CT) scan was requested for further evaluation.

Contrast-enhanced chest CT showed a voluminous (60 × 60 × 44 mm; estimated volume 83 mL), thick-walled, loculated cyst in the left pleural space. There was moderate parietal enhancement.[21] The liquid contents in the cyst showed a homogeneous density.[22] The surrounding lung contents were mostly collapsed.[23] The mediastinum was displaced to the right side; consequent pressure effects compressed the parenchyma of the right lung and caused diffuse ground glass opacity. A moderate pleural effusion was noted on the left side. The cyst was considered as likely to be bronchogenic in origin (Fig. 2).

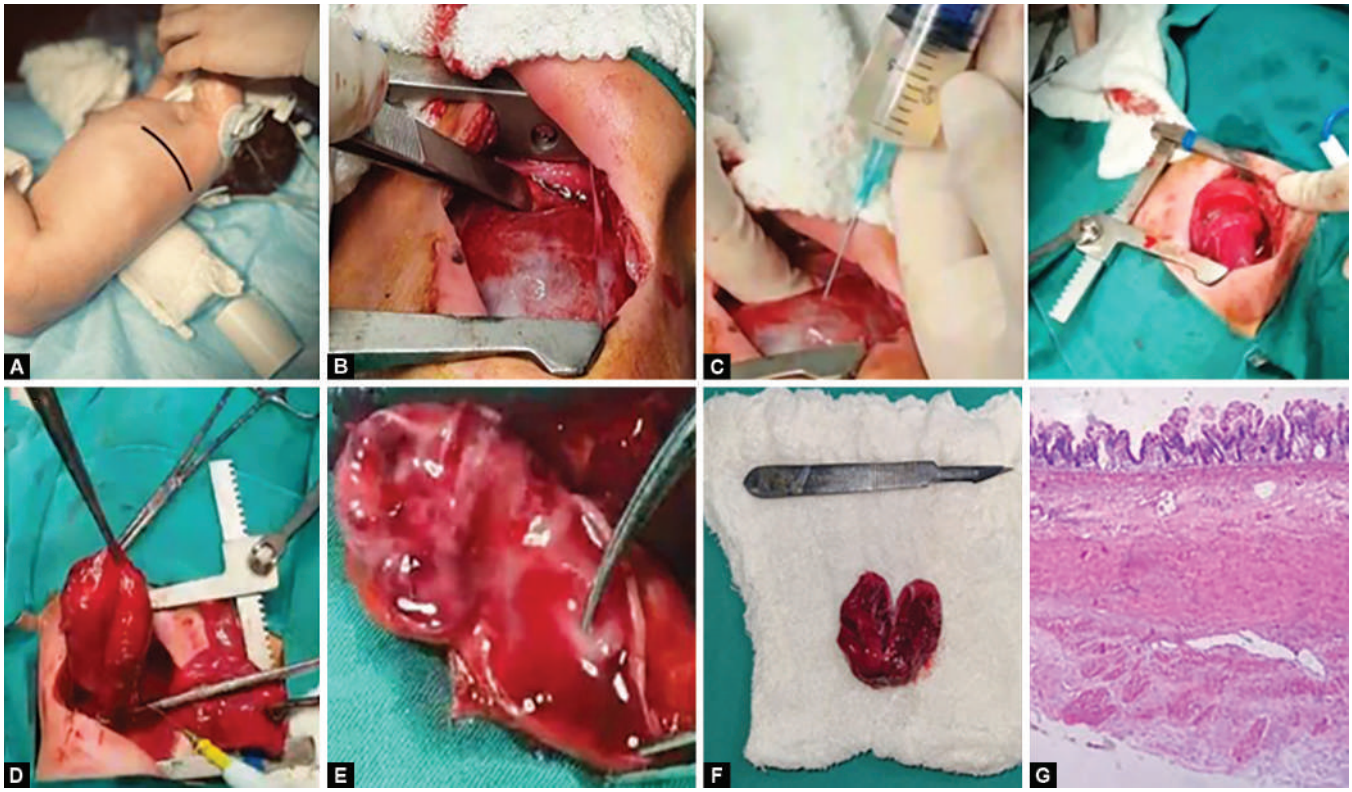
A left posterolateral thoracotomy was performed on postnatal day 8. A giant cystic tumor was dissected; there was a tense capsule covering the entire pleural cavity with firm adherence to the lower lobe, pulmonary apex, posterior pleura, esophagus, descending aortic artery, and diaphragm. The surrounding atelectatic lung tissue was largely intact in structure. A pleural drain was placed. The histopathological examination of the cyst wall showed features of gastric and intestinal mucosa with a thickened *muscularis propria* (Fig. 3). The postoperative hospital course showed good recovery without complications. Later chest X-rays did not show any abnormalities (Fig. 4). The infant was discharged 3 weeks after surgery.

Discussion

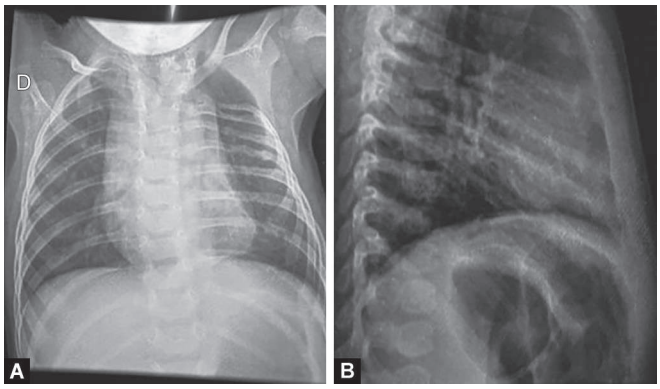
This case is important as it represents a not-so-unusual situation where we, as pediatricians/neonatologists, may have our first interaction with the mother in the delivery/surgery room and have not had complete information about the prenatal course and investigations.[24] Such deficiencies in prenatal care of the mother-infant dyad are a recognized issue in quality of care all over the world and need attention.

The chest radiograph obtained in the neonatal unit showed a massive cardiomegaly; the clinicians have traditionally considered such radiograph findings as likely due to an Ebstein's anomaly or a large pericardial effusion. Echocardiography was not available in the unit during the night shift, and so MR and CT scans were done. MR showed that the enlarged cardiac silhouette that we had seen on the CXR actually contained a large thoracic cyst, which was possibly bronchogenic or gastrointestinal in origin. There was no cardiomegaly or a pericardial effusion large enough to explain the mediastinal enlargement. CT provided additional structural details of the cyst and secondary atelectasis of the surrounding left lower lobe. These findings emphasize the utility of timely, advanced imaging when radiographic findings are concerning but inconclusive.

In our infant, the histopathological examination of the lining of the intrathoracic cystic mass showed features of both gastric and small intestinal mucosa. These clinical-radiological-histopathological findings have been described as bronchopulmonary foregut malformations (BPFMs). These anomalies are believed to result from anomalous budding of the foregut and tracheobronchial tree in the developing embryo.[25,26] These malformations have been classified into three categories: (a) congenital pulmonary airway malformations; (b) pulmonary sequestrations; and (c) foregut duplication cysts such



Figs 3A to G: Surgical excision and histopathological evaluation. (A and B) Left posterolateral thoracotomy. The presence of a giant tumor with a cystic appearance with a tense capsule covering the entire pleural cavity with firm adherence to the lower lobe, pulmonary apex, posterior pleura, esophagus, descending aortic artery, and diaphragm; (C) Drainage of serous fluid from the cyst; (D) Successful surgical dissection; (E) Left lung intact, pleural drainage tube was placed; (F) Excised cystic tumor; (G) Histopathological findings: hematoxylin & eosin-stained tissue section (10×) shows features of gastric and duodenal-like lining, and thickened muscularis propria



Figs 4A and B: Anteroposterior chest X-ray showing preserved lung expansion at the time of discharge

as bronchogenic, neuro-enteric, and enteric cysts.[27] The mucosal lining in these cysts is often heterotopic and may not resemble that in the adjacent bowel. The ectopic gastric mucosa is seen in 20–30% of cases, possibly more often in esophageal and midgut duplication cysts. Gastric duplications may show pancreatic mucosa. Bronchogenic cysts may show respiratory epithelium, cartilage, and bronchial submucosal glands.

Gastrointestinal duplication cysts, similar to the findings seen in our patient, are rare congenital anomalies found in about 0.2% of infants, in about 1 in 4500 births.[28,29] A slight male preponderance has been observed. Most of these patients present during infancy.[30,31] Enteric duplication cysts typically show three characteristics: (a) proximity to segment(s) of the gastrointestinal tract (GIT); (b) a well-developed smooth muscle coat; and (c) mucosal lining found within some portion of the GIT.[32] Many cysts show a distinct spherical or tubular appearance. Spherical cysts are seen (80%) more frequently than tubular duplications, which communicate with the bowel lumen.

The exact etiology of gut duplication cysts is unknown, but some embryological aberrations between 4 and 8 weeks' gestation may be associated. A split notochord, partial twinning, persistent embryological diverticula, and aberrant luminal re-canalization could be some of these possibilities.[33,34] The split notochord theory suggests that the growing notochord may split earlier/incorrectly from endodermal cells. These events could explain the co-occurrence of vertebral anomalies, as in our infant, with duplication cysts.[35] Intrauterine trauma or hypoxia could be contributing factors.

The recanalization theory emphasizes that altered recanalization of the gut may manifest as these cysts.[33] However, the occurrence of duplications in segments that do not undergo recanalization during embryological development is difficult to explain. The embryonic diverticula hypothesis emphasizes abnormal persistence of embryonic diverticula in the alimentary tract. The presence of heterotopic mucosa in these cysts cannot be explained.[33] The partial or abortive twinning theory emphasizes incomplete twinning of the developing gastrointestinal tract. The association of doubling

anomalies of the genitourinary tract with colorectal duplication cysts may support the theory, but not the occurrence of these cysts in other areas.

Intrathoracic cystic lesions pose a diagnostic as well as a therapeutic challenge; as in our case, the CXR may just show an enlarged mediastinal silhouette that needs further evaluation.[36] Antero-posterior and lateral views can help, but obtaining a lateral view is not a routine procedure in most neonatal units.[37] In some cases, point-of-care ultrasound could be helpful.[27] The detection of spinal abnormalities should raise the suspicion of neuro-enteric cysts arising from the thecal sac.[38] As in our case, CT or MR imaging is useful.

Early surgical resection of intrathoracic cysts can be helpful. Progressive enlargement of the cyst can compress the bronchi and smaller airways, affecting airflow and lung parenchyma.[39,40] Secondary infections can be seen.[39,41,42] Thoracoscopic excision can be performed with skilled hands; there can be difficulties in small infants and large-sized cysts.

In conclusion, we need to remember that not every massive cardiomegaly in a newborn infant is due to an Ebstein's anomaly or a large pericardial effusion. Other diagnostic entities, such as BPFMs, should be included as a differential diagnosis during the assessment of a mediastinal mass.

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CASE REPORT

Two Novel Mutations Associated with Familial Chylomicronemia in a Neonate

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Abstract

We recently treated a 12-day-old male infant who was presented with respiratory distress, hepatosplenomegaly, and *lipemia retinalis*. The laboratory notified us that his blood samples were unusually viscous and pinkish-white and turned opaque milky white in about 10 minutes. The acute phase reactants were consistent with inflammation but the cultures remained sterile. Sera showed chylomicronemia with high triglyceride and cholesterol levels. We changed feedings to a special formula containing medium-chain fatty acids. Genetic analysis showed a novel homozygous mutation in the lipoprotein lipase (LPL) gene. In addition, he had a heterozygous missense variation in the sterol regulatory element-binding transcription factor 2 (SREBF2) gene. His father was also found to have hypertriglyceridemia and is being evaluated. This case reminds us yet again that not every infant with respiratory distress has an infection as the underlying cause. Timely diagnosis and intervention can improve outcomes.

Keywords: Case report, Consanguineous marriage, Hepatosplenomegaly, Infant, Lipoprotein lipase, *Lipemia retinalis*, Medium-chain fatty acids, Neonate, Newborn, Respiratory distress.

Newborn (2024): 10.5005/jp-journals-11002-0105

Keypoints

- Familial chylomicronemia (FC) usually presents in neonates with respiratory distress, hepatosplenomegaly, and increased blood levels of chylomicrons. The retinal blood vessels are opacified due to lipemia. Blood samples turn milky white due to high triglyceride and cholesterol levels.
- Familial chylomicronemia is an autosomal recessive disorder. We recently treated a 12-day-old infant for respiratory distress. He turned out to have a hitherto undescribed mutation in the lipoprotein lipase (LPL) gene. In addition, he also had a heterozygous missense variation in the sterol regulatory element-binding transcription factor 2 (SREBF2) gene.
- The infant responded to a change in feedings to a special formula containing medium-chain fatty acids.
- This case reminds us of the need to be cognizant of non-infectious causes of neonatal respiratory distress. Timely diagnosis and intervention can improve outcomes.

Introduction

We recently evaluated and treated a 12-day-old male infant with respiratory distress, hepatosplenomegaly, and opacified retinal vessels (*lipemia retinalis*).[1,2] There was no evidence of sepsis or a viral syndrome.

He had significantly high plasma total triglyceride (TG) and total cholesterol (TC) levels.[3] These clinical findings were consistent with those seen in familial chylomicronemia syndrome (FCS).[4–7] In the following sections, we provide full clinical and genetic details. This case is a reminder that we need to carefully evaluate late-onset neonatal respiratory distress not only for infectious but also for non-infectious causes. Timely diagnosis and intervention can improve outcomes.

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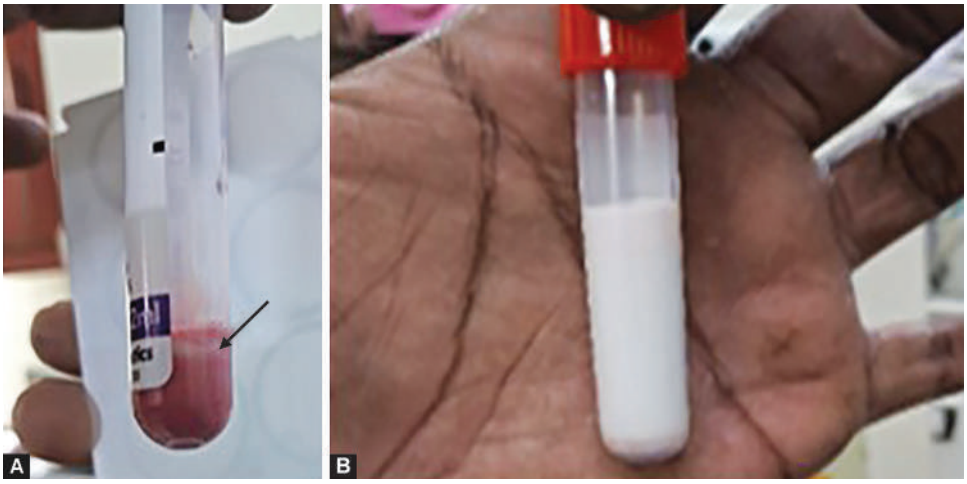
Case Description

A 12-day-old male infant was recently admitted in our neonatal unit with respiratory distress. His mother was a primigravida (G1P0) and the antenatal period was uneventful. The only notable finding in the records was a 2nd degree consanguineous marriage of the parents. The infant was born by vaginal delivery at 38 weeks' gestation with a birth weight of 2800 grams. Apgar scores were 8, 8 at 1 and 5 minutes, respectively; no active resuscitation was needed. There were no high-risk factors for early- or delated-onset sepsis or viral/bacterial infections. The infant was exclusively breastfed since birth.

At admission, the infant was tachypneic (respiratory rates of 70/min) with mild retractions. The lower edge of the liver was palpable 4 cm below the right costal margin. The spleen was not palpable.



Figs 1A and B: Newly obtained blood sample was viscous and showed a red-white appearance with white bands (arrow) (A). The supernatant looked hazy; (B) After 10 minutes, the sample became completely milky white

The lungs looked unremarkable on radiographs. Ultrasound examination of the abdominal confirmed hepatomegaly and also showed a mild splenomegaly.

To investigate the cause of respiratory distress, a complete blood count (CBC) and blood cultures were obtained. The blood sample collected for CBC (anticoagulant: ethylenediaminetetraacetic acid, EDTA)[8] was viscous and reddish-white in appearance. The supernatant looked hazy. The whole sample turned milky white in 10 minutes (Fig. 1). This change of serum samples turning creamy white during storage, the "refrigerator test," has long been viewed as evidence of elevated blood chylomicron (CM) levels in FCS (type I hyperlipidemia).[8,9] The total/differential leukocyte and platelet counts were unremarkable. C-reactive protein levels were high (21 mg/dL; normal 0–6 mg/dL) but the blood and urine cultures showed no growth.[10,11] The serum lipid profile showed markedly elevated TG levels of 4425 mg/dL (normal <295 mg/dL) and high TC levels of 705 mg/dL (normal <200 mg/dL); the TG/TC ratio of 6.28 was higher than the diagnostic threshold of 5 typical of FCS. Fundal examination showed signs of *lipemia retinalis* (Fig. 2).[2]

Considering a diagnosis of dyslipidemia, we started feeding the infant with a special formula containing medium-chain TGs. Later, we added vegetable oil for caloric supplementation and also started low-fat (skimmed) cow's milk per his parents' wishes. The infant showed a good clinical response to these dietary changes and became asymptomatic in 5–7 days. A lipid profile repeated 2 weeks later showed all values within normal limits.

To investigate the origin of the altered lipid profile of the infant, we next examined the lipid levels in the parents. His father had serum TG levels of 1332 mg/dL but the cholesterol levels were normal (132 mg/dL). Maternal samples showed normal TG levels (279 mg/dL). Her cholesterol levels were elevated at 369 mg/dL but a repeat test showed levels within normal limits. Based on these findings, we considered the serum lipid abnormalities seen in the infant as originating from the father.

Genetic analysis of the infant showed abnormalities in the lipoprotein lipase (LPL)[12] and the sterol regulatory element-binding transcription factor 2 (SREBF2; also described as the sterol regulatory element-binding protein 2, SREBP-2)[13] genes (Fig. 3). In LPL (chromosome 8p22),[14] there was a homozygous[15] missense mutation[16] (codon 215, GGG to GAG) in exon 5; this resulted in amino acid substitution of glycine with glutamic acid (Fig. 4). His father was heterozygous[15] for the same mutation. The maternal LPL showed no abnormalities. The infant showed another heterozygous missense variant[16] in exon 11 of the sterol regulatory element-binding transcription factor 2 (SREBF2; also described as the sterol regulatory element-binding protein 2, SREBP-2; chromosome 22q13.2).[13,14] This change in sequence (codon 69, AUG to GUG) indicates a substitution of methionine with valine. Genetic analysis of the parents for SREBF2 was ambiguous and is being repeated.

Discussion

Our infant showed findings typical of FCS, including the "positive" refrigerator test suggestive of high serum levels of CMs; TG and TC levels; hepatosplenomegaly; and *lipemia retinalis*.[9,17] As we know, CMs comprise one of the four major classes of lipoprotein particles; the other three being the very low-density lipoproteins (VLDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs).[18] FCS is characterized by high serum TG and TC levels similar to the findings in our patient as these are the two major constituents of the CM core (75% TGs, 25% cholesterol). The covering phospholipid membrane contains apolipoproteins (2% weight) and cholesterol.[19] The size of CMs ranges between 75 and 600 nm, or even more, increasing with the incorporation of dietary TGs, fatty acids, and cholesterol.[19]

Our patient showed a novel homozygous missense Gly^{215}→Glu mutation in exon 5 of the LPL gene. Loss-of- function mutations[16,20,21] in the LPL gene are a frequent cause of FCS.[20,22–27] Lipoprotein lipase deficiency is seen with an incidence of about 1 in a million; some ethnic variations have been reported.[5,6,26,28,29] The inheritance is autosomal recessive most patients with symptomatic disease usually have loss-of function mutations in both copies of the gene in important cell lineages/organs.[30] Some FCS patients with LPL deficiency are compound heterozygotes;[31] mutations in exons 4, 5, and 6 of the LPL gene frequently result in a catalytically inactive LPL enzyme protein.[22,25] Others are homozygous for two-point missense mutations[16] in the LPL gene (Asp^{9}→Asn, Tyr^{262}→His, Asn^{291}→Ser, Trp^{86}→Arg, Gly^{188}→Glu, Pro^{207}→Leu, and Asp^{250}→Asn, which alter the stability of the LPL dimer.[22,24,27,32–34] Overall, the Gly^{188}→Glu mutation is the most frequent mutation in patients with

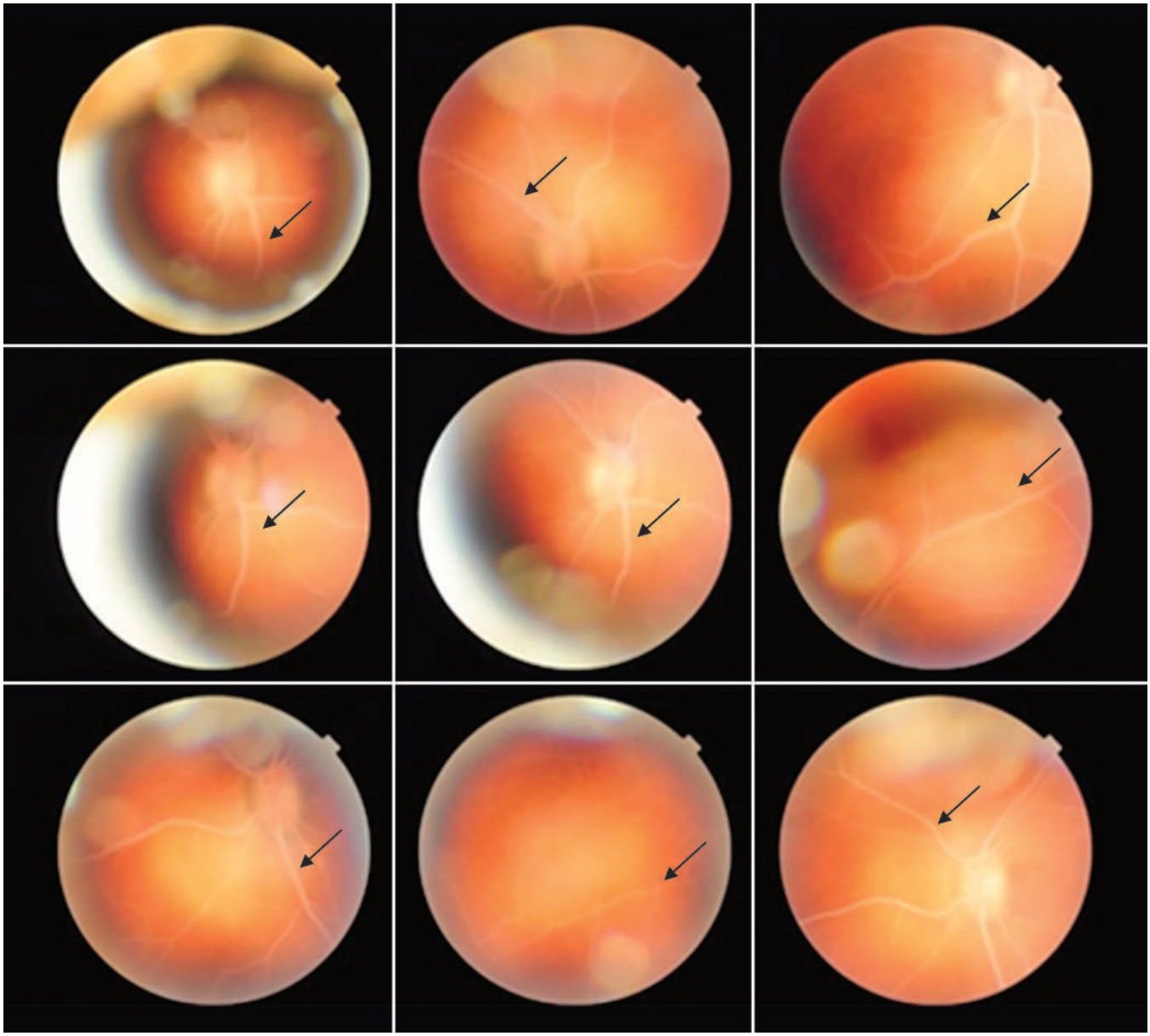
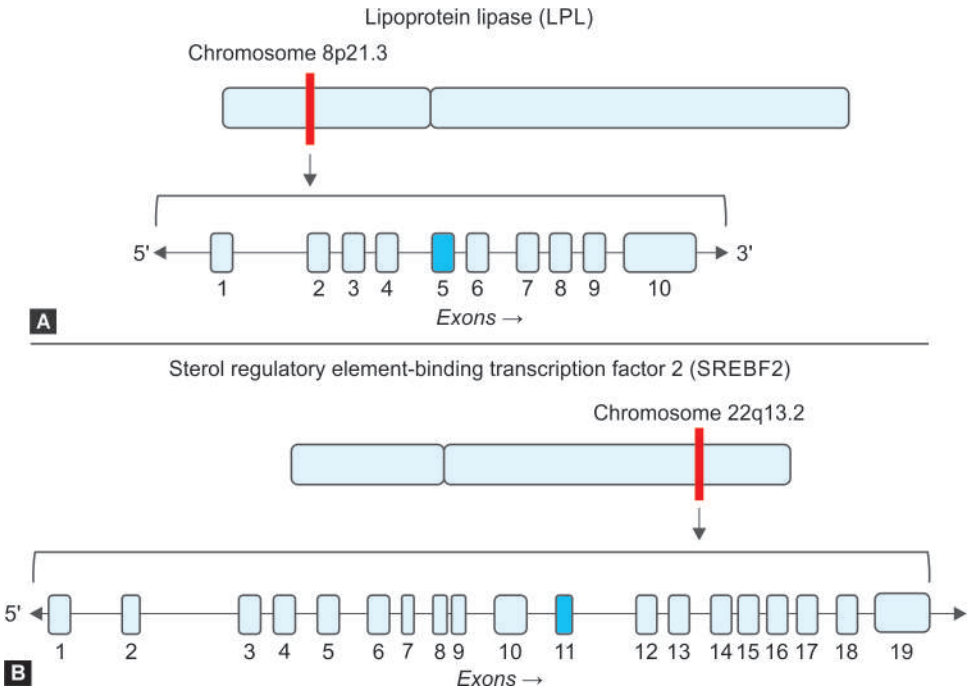


Fig. 2: Fundus showed *L. retinalis* with creamy white retinal vessels (arrows). These findings are typically associated with high levels of triglycerides in blood due to deficiency of lipoprotein lipase

chylomicronemia and LPL deficiency in the general population.[23,35] Noting further, the serum TG level in our patient was 4425 mg/dL, which was much higher than the 1332 mg/dL level measured in his father. His mother's levels were normal.

Our patient had a homozygous missense mutation,[16] which likely occurred occur due to uniparental disomy[36] where the chromosome pair was derived from one genitor, or from an imprinting disorder.[37] When the uniparentally derived pair carries two homozygous sequences (isodisomy) with a duplicated mutant, this "reduction to homozygosity" can lead to a recessive phenotype solely inherited from one heterozygote.[38,39] A loss-of-function mutation in the SREBF2 gene could also have contributed to his clinical findings (described below).

Lipoprotein lipase gene is mapped to human chromosome 8p22 and is comprised of 10 exons spanning about 30 kb[26] (Fig. 3). The first exon encodes the 5′-untranslated region, the signal peptide plus the first two amino acids of the mature protein. The next eight exons encode the remaining 446 amino acids, and the 10th exon encodes the long 3′-untranslated region of 1,948 nucleotides.[40] Mature, functional LPL protein is a homodimer; the two protein molecules are anchored by heparan sulfate proteoglycans and/or glycosyl phosphatidylinositol to the vascular surface of the endothelium.[41] Each of these proteins has two structurally distinct domains, an amino-terminal and a smaller carboxyl-terminal domain, connected by a flexible peptide.[42] The amino-terminal domain contains the catalytic triad involved in lipolysis (Ser^{132}, Asp^{156}, and His^{241}).[43] The carboxyl-terminal domain contains the dominant heparin-binding domain that binds lipoproteins.[44] LPL is expressed in a variety of cells, including the adipose tissue, cardiac and skeletal muscle, pancreatic islets, and macrophages.[45] Functionally, it



Figs 3A and B: Schematic illustrations of the LPL and SREBF2 genes mark the abnormal exons (depicted in black) in our index case. We found (A) A homozygous missense mutation in exon 5 of the LPL gene; (B) There was also a heterozygous missense variant in exon 11 of the SREBF2 gene

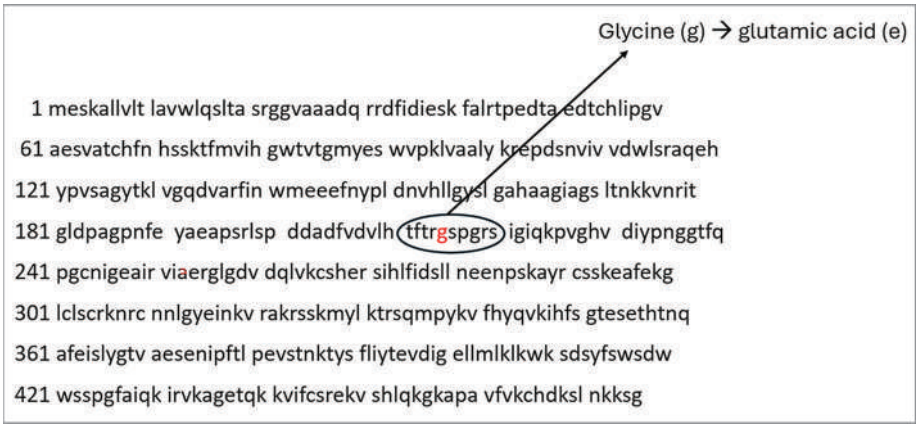


Fig. 4: The genetic mutation in the LPL gene in our index case is consistent with amino acid substitution of the glycine residue (g, red font) on position 215 with glutamic acid (e)

is the rate-limiting enzyme for the hydrolysis of the TG core of circulating chylomicrons and other lipoprotein lipids.[45] The enzymatic activity is regulated in a complex manner in response to energy requirements and hormonal changes. Increasing evidence suggests that LPL is regulated at transcriptional, posttranscriptional, translational, and posttranslational levels in a tissue-specific manner.[45]

In CMs, LPL can promote the exchange of lipids between lipoproteins, receptor binding, and the cellular uptake of these particles with associated lipids and lipophilic vitamins.[18,41,46,47] It interacts with the apo-B48 protein molecule in the CM membrane to promote CM assembly in a feed-forward mechanism and subsequent transport via lymphatics and the systemic circulation.[48,49] This protein is stabilized by the lipase maturation factor 1 (LMF1) on the endoplasmic reticulum (ER) and is then secreted.[50] The protein glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) is a key co- factor in this process.[41] Lipoprotein lipase is also attached to enterocyte-derived particles containing a microsomal triglyceride transfer protein (MTP).[18] On the CM covering, apo-CII is another specific cofactor that anchors LPL to the endothelium.[51] In this list, apo-A5 is another activator of LPL.[52] FCS has been associated with mutations in these four genes, namely, LPL, LMF1, apo-CII, and apo-A5.[53]

Blom et al.[54] have previously described a cohort of 66 patients with FCS. The mean ± standard deviation age was 46 ± 13 years. They identified causal mutations in 52 (79%) of their patients; LPL mutations accounted for 41 (62%) cases. The median age at diagnosis in their cohort was 24 years, where 54% were females, and 81% were Caucasian. Similar to this cohort, the detection of hepatosplenomegaly, *lipemia retinalis,* and altered TG and TG/

TC ratios in our patient were all consistent with the diagnosis of FCS.[55,56] Our infant did not show any skin lesions such as eruptive xanthomas, which have been seen in some patients.[57] The detection of LPL mutations confirmed the diagnosis. One difference is that we detected a mutation in exon 5, a finding that differs from previous reports of alterations in exon 3.

As in our patient, dietary modifications can help in the reversal of symptoms of FCS.[17,58] Most patients respond to restricted low-fat diets of less than 15% of the calories (20 gm/day) coming from fat and MCTs. The mainstay of management is strict adherence to fat restriction with a low-fat formula.[59] Fat restriction which should be continued throughout life.[59] The addition of MCTs has also been used for patients with chylomicronemia as these are directly absorbed into the portal circulation.[17] In this condition, traditional TG-lowering agents, including fibrates, niacin, statins, and fish oil, are not effective.[60] Some therapies currently in development include LPL gene therapy and RNA interference of apo-CIII.[61,62] Agents such as lomitapide have been used to inhibit MTP in adults but have not been useful for FCS.[63]

We also detected a Met^{69}→Val mutation in the SREBF2 (SREBP2) gene. Loss-of-function mutations/protein function in SREBF2 could have contributed to the inflammatory response (respiratory signs, high C-reactive response), and the elevated TG and cholesterol levels in our patient.[64] The parents of our infant showed normal cholesterol levels, suggesting that altered sequence we noted in the SREBF2 gene in our infant could be a *de novo*[65] mutation. Sterol regulatory element-binding transcription factor 2 is a key regulator of genes involved in cholesterol biosynthesis.[66] Its own expression is regulated in a negative feedback loop triggered by sterols in the ER.[67] The precursor protein of the SREBPs binds SCAP (SREBP-cleavage activating protein), which also regulates SREBP maturation in the ER.[68,69] SCAP promotes the translocation of SREBP from the ER to the Golgi apparatus, allowing it to regulate cellular TG and cholesterol levels.[70] The activation of SREBPs is regulated by a negative feedback loop triggered by sterols in the ER.[71] When the levels of cellular cholesterol are high, the SCAP-SREBP complex is maintained at high levels in the ER.[69,71] High levels of sterol alter SCAP conformation.[72] These factors promote the transport of the SCAP-SREBP complex from the ER to Golgi, where these proteins are cleaved.[70] Cleaved SREBPs release the transcriptionally active NH_2-terminal domains, that can enter into the nucleus and form a positive-feedback transcription loop and consequently, induce the aforementioned downstream changes.[71,73] SREBP can also alter macrophage polarization and promote the resolution of inflammatory signaling by reprogramming fatty acid metabolism.[71,73–76]

To summate, we found this case as a reminder that not every infant with a systemic inflammatory response syndrome has sepsis as the inducing factor.[6] We need to remain cautious that some cases might have etiological factors other than infections, and timely diagnosis and treatment can help. We will continue to follow this infant for long-term changes.

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