October–December 2025 Volume 4 Issue 4 newborn Official Journal of the Global Newborn Society eISSN: 2769–514X

newborn

October-December 2025
Volume 4
Issue 4
eISSN 2769-514X

Official Journal of the Global Newborn Society and 53 allied organizations
International Society for Marginalized Lives
Dr. Mozib Newborn Foundation
The Carlo GNS Center for Saving Lives at Birth
Vishwa Mahesh Parivaar
Autism Care Network Foundation

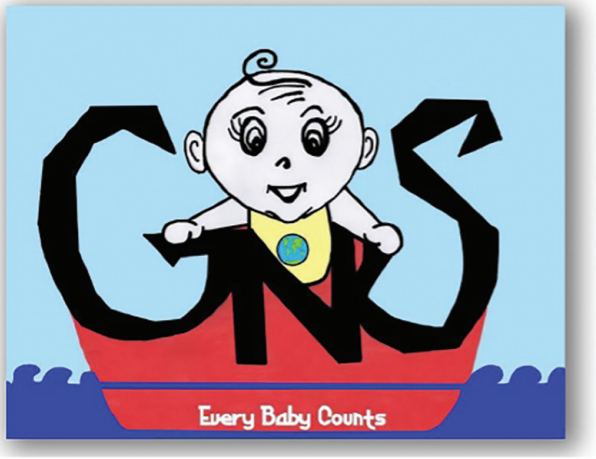
And
GNS Down Syndrome Foundation
Newborn Foundation of Azerbaijan
GNS Bangladesh Newborn Foundation
GNS Foundation of Germany
Global Newborn Society Foundation of Italy
Mongolian Association of Obstetrics Gynecology and Neonatology
Foundation for Human Milk Feeding in the Islamic World
The organization, Protecting Brains and Saving Futures, Brasil
Association of Neonatologists in the United Kingdom
Polish Nursing Association - Płock, Poland
Panlibyan Neonatal Association
Association for Indigenous Peoples in India
Association for Newborn Care in Pakistan
GNS Association for Perinatal Care
Association for Infant Nutrition in the Middle East
Sociedad Latinoamericana de Residentes de Neonatologia (SolaReNeo)
Uruguayan Neonatal Association
Paraguayan Society of Pediatrics Committee for Neonatology
Armenian Association of Neonatal Medicine
Association of Pediatricians of Uzbekistan
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
Dudeja GNS Center for Infectious Diarrheal Diseases
Anatolian Midwives Association
GNS Western Australia
Perinatal Society of Singapore
Pioneers - looking for sustainable ways to reduce infant mortality
Bhutan Neonatal Care Forum
Global Newborn Society Iran Chapter
National Federation of Neonatologists of Mexico
College of Neonatologists of the State of Jalisco, Mexico
The Skylar Project
International Society for Marginalized Lives
Friends Aid Africa, Bukedea, Uganda
Society of Bacteriophage Research and Therapy
GNS Center for Computational Scientific Methodology
GNS International Association of Neonatal POCUS
SABREE Enrichment Academy: Empowering Ability
The Caribbean Association for Hematology and Oncology
First Breath of Life
GNS Neonatal Radiology Forum
Global Newborn Society, Orthopedic Surgery Section

Highlighted articles:

Systematized Systemic Sonographic Surveillance (S4)
A High-Throughput Analysis of Gene Expression in the Intestine in Severely-Anemic Mouse Pups
Advances in Viscoelastic Coagulation Monitoring

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.

Issue Information

Volume 4, Issue 4; October–December 2025

eISSN: 2769-514X

Published: GNS, LLC; 6114 Lily Garden, Clarksville, MD, USA; Ph +1 708 910 8729

Printed: Jaypee Brothers Medical Publishers
4838/24, Ansari Road, Daryaganj, New Delhi 110 002, India
Phone: +91 11 4357 4357, Fax: +91 11 4357 4314

Contents

Editorial

Big Ideas Start Small ..... **iv**
Ling He, Sonji Fatima (Daniel) Harold, Adrianna Frydrysiak – Brzozowska

Original Research

Screening for Developmental Dysplasia of the Hip in Saudi Arabia: Technically Feasible, but Social Barriers Persist **159**
Naief Alghnimei, Yaquob Daghriri, Attallah Alhwiti, Nayef Alsharari, Ahmed Eltayeb, Duha Alshama, Mohammed Gharib, Malak Almehmadi, Yousef Alshammari, Saif Alanazi, Meshari Alhawiti, Abdulrahman Alelyani, Abdullah Alayda, Zaben Alanazi, Ali Daghriri, Yahya Ethawi

A High-throughput Analysis of Gene Expression in the Intestine in Severe Neonatal Anemia **165**
Jayanta K Das, Akhil Maheshwari

Clinical Technique

Clinical Procedures: Use of Peripherally Inserted Central Catheters in Neonates **178**
Monika Kaushal, Kalyan C Balla

Systematized Systemic Sonographic Surveillance (S4) **181**
Srijan Singh, Monika Kaushal, Brunetta Guaragni, Salvatore Aversa, Naief Alghnimei, Md Rezaul Hayat, Ayush Kaushal, Ghania Daar Ede, Jeremías PGB Duré, Jargalsaikhan Badarch, Md Mozibur Rahman, Ola D Saugstad, Rachana Singh, Akhil Maheshwari

Review Articles

Ethical Challenges in Neonatal Life Support **194**
Srijan Singh, Sherri S Buddington, Roya Huseynova

Maternal Determinants of Neurodiversity in the Developing Brain: An Integrative Review of Biological, Environmental, and Psychological Factors **203**
Chhaya S Prasad, Akhil Maheshwari

Case Report

Case Report: A Congenital Pouch Colon with Anorectal Malformation and Associated Anomalies **221**
Atul K Khare, Ramesh C Tanger, Aditya J Baindur

Short Communication

Advances in Viscoelastic Coagulation Monitoring **225**
Brunetta Guaragni

10.5005/newborn-4-4-iv

Big Ideas Start Small

The 1st conference of the Global Newborn Society (GNS) was held at the Nobel Prize Museum, Stockholm[1] and Uppsala University, Sweden[2] on November 2–4, 2025.[3] It turned out to be a well-attended event with many physicians, nurses, and social leaders,[4] and provided a fresh spark for a rapidly-growing organization. We now have more than 10,000 members from all the 6 populated continents. It has indeed been a nice trajectory for an organization that started merely 4 years back.[5]

We all know that every breakthrough begins as a simple thought.[6] Neonatal mortality has decreased over the last 50 years,[7] but we still need an organization that will truly span the globe to cover gaps in care and outcomes of newborn infants.[8] We still have had to call most ailments in neonates as syndromes, not diseases.[9] A syndrome is a cluster of clinical features where the exact cause is unclear. The treatment, therefore, is focused on the management of pathophysiological deficits, with a hope that ongoing post-conceptional maturation will help. In contrast, a disease is a specific medical condition with a clearly-identified cause, such as an infection, genetic defect, or organ malfunction; it can accurately diagnosed using specific tests and then treated accordingly. Neonatal syndromes occur with different frequencies in different parts of the world,[10] and might even have different names![11] We have all had this longing for a platform – call it the way we may want to – a question, a sketch, or a first step taken without certainty. But we also know that small actions can build momentum, turn curiosity into confidence, and effort into impact.[12] We hope that ideas will be tested, refined, and strengthened.[13] What matters most isn't how big the idea is at first, but the willingness to begin and keep going.

There was a range of ideas with a variety and diversity of thoughts, perspectives, and solutions. This made the meeting exciting; there were both ideological thoughts - carefully considered, well-reasoned views that reflected awareness and experience; and thoughtful ideas with insights into the context, consequences, and needs. Not surprisingly, the combination sparked exciting discussions. All of us long to find transformational ideas for better thinking. These might begin as seemingly-simple insights but could then reshape individual and system-wide behavior. What makes an idea truly transformational is not just its originality, but its ability to create meaningful change that reaches beyond the initial spark.[14]

To ensure safety, the Nobel Museum caps the number of attendees at a single event at a relatively small number.[15] These restrictions in the inauguration event did limit the number of invitees in the subsequent days of the conference, but for our newly-founded global organization, the very possibility of access to these renowned historical sites inspired aspiration. The Nobel Prize Museum needs no introduction.[16] And neither does Uppsala University, the land of Carl Linnaeus,[17] Anders Celsius,[18] Svante Arrhenius,[19] and Dag Hammarskjöld.[18, 20] The central geographical location (close to the Prime Meridian[21]) also made it relatively easy for the participants to join without major time-zone lag-related fatigue.[22] The Schengen visa process was also perceived as well-organized by the attendees. Many experts from Africa, South America, and Australia joined online, and we had participants from all over the world **(Fig. 1)**.[23]

We would like to further congratulate our members. The Global Newborn Society continues to grow—a new suborganization has emerged in the GNS in recent months. Dr. Naief Alghnimei and colleagues from the Kingdom of Saudi Arabia have formed a Global Newborn Society Orthopedic Surgery Section. They have expertise in surgical management of orthopedic anomalies in newborn infants, and are also actively looking for genetic causes/associations. This multispecialty effort adds a new dimension to the GNS. This is the 53rd allied group that has adopted this journal as its official mouthpiece.

In each issue of the newborn, our editorial team highlights the achievements of one of our partnering members. Here, we present the efforts of the Society of Bacteriophage Research and Therapy is headquartered in Varanasi, India.[24] This organization is emerging as an leader in our understanding of the impact of bacteriophages on host immunity and environmental bacterial contamination. Bacteriophages are viruses that infect and reproduce inside bacteria.[25–33] These attach to a bacterial cell, inject genetic material, and use the bacterium's molecular machinery to make new phages **(Fig. 2)**.[34] Bacteriophages are highly specific, usually infecting only one type or strain of bacteria, and can possibly play an important role in controlling bacterial populations.[35]

This journal aims to cover fetal/neonatal problems that begin during pregnancy, at the time of birth, or during the first 1,000 days after birth. As in our previous issues, we present 8 articles here **(Fig. 3)**. Singh and her team[36] continue to develop the systematized systemic sonographic surveillance (S4) program[37] as a structured,[38] sonographic technology-enabled extension of the physical examination of newborn infants. The philosophy of S4 differs from point-of-care ultrasound (POCUS),[39] which is known for post hoc bedside confirmation/assessment/temporal monitoring of organ-system injury in at-risk/critically-ill patients. S4 utilizes radiation-free, real-time imaging for *a priori* assessment of risk or detection of sub-clinical lesions. They examine the brain, heart, lungs, liver, bowel, urogenital tract, spine, and hips. They are also using sonographic guidance in placement of central lines and for lumbar punctures. This system may improve training, clinical decision-making, and possibly, neonatal intensive care outcomes.[40]

Alghnimei et al.[23] have shown that even though screening for developmental dysplasia of the hip joints is technically feasible in Saudi Arabia, social barriers still persist. They performed a retrospective observational cohort study in Saudi Arabia; they followed a cohort of 3598 infants; 95 were identified as high-risk for DDH. These infants were followed with at least 1 in-person and then via direct clinical and/or telephonic contact. A standardized questionnaire was used to understand parental perception and factors affecting compliance with the screening process. The authors were able to follow 47/95 (49.5%) infants. The other 48 at-risk patients could not be followed; 36 (76.6%) could not be reached because of incorrect contact information, 11 (23.4%) families were not aware of the importance of timely diagnosis and close follow-up, and 13 (28.6%) could not reach the clinic because of difficulties in access to medical

Figs 1A to C: *Continued*

Contd...

Figs 1A to C: *Continued*

Contd...

Figs 1A to C: *Continued*

Contd...

Figs 1A to C: *Continued*

Contd...

Figs 1A to C: *Continued*

Contd...

Figs 1A to C: (A) The opening session was held on November 2, 2025, at the Nobel Prize Museum in Stockholm, Sweden (location seen in the first two images). Academic and social leaders from all over the world attended the event. Many shared their views electronically; (B) The conference sessions were held on November 3 and 4 at the Uppsala University (location seen in the first two images). Leading experts from 70 countries shared their experiences in presentations and discussions in the central Aula auditorium and adjoining conference rooms; (C) A concluding session was held for feedback and planning for future conferences. Many of these presentations have been uploaded on social media sites such as YouTube after permission from the experts

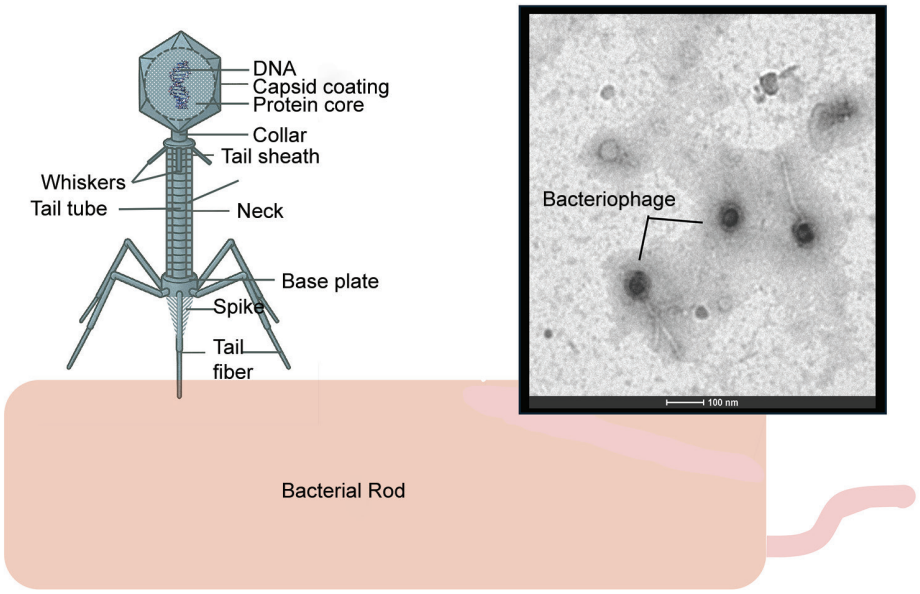


Fig. 2: Schematic showing a bacteriophage attached to a bacterial rod. Inset shows an electron micrograph showing bacteriophages

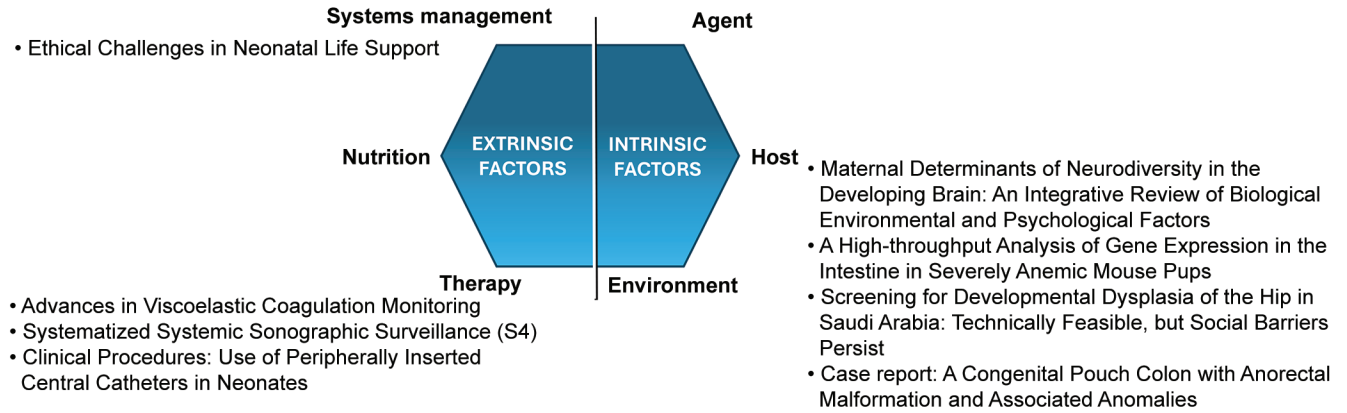


Fig. 3: Areas of focus in the *newborn*, Volume 4, Issue 4. We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health - those originating in therapy, nutrition, and systems management are shown. This issue covers 3 nodes, with articles focused on host factors, therapy/monitoring systems, and systems management

support because of long distance/difficult terrain. Others either did not believe in the need for screening - 2 (4.2%) or missed their appointment (4, 8.5%). There is a need to strengthen efforts for clinician and parental education regarding DDH.[41]

Prasad and Maheshwari[42] have reviewed maternal determinants of neurodiversity in the developing brain.[43] Neurodevelopmental delay has been a major clinical concern but a view is now emerging that we need to consider the possibility of neurodiversity, a natural variation in cognitive and behavioral traits observed across individuals.[44] Many differences in the human brain may originate during early development, when biological/environmental signals shape the fetal neural architecture and consequently, manifest as a spectrum of thinking, learning, attention, and behavior during childhood and later.[45] Conditions such as autism, attention-deficit/hyperactivity disorder, and dyslexia might need to be viewed as alternative ways of experiencing and interacting with the outside world.[46] This diversity model is a paradigm-shift in how we currently view these infants; it will call for acceptance, inclusion, and support in the society, schools, and workplaces to value diverse cognitive strengths.

Das and Maheshwari[47] used their established mouse model and microarrays for an open-ended, high-throughput approach to determine the impact of severe anemia on the intestinal mucosa on the genetic level. They have previously reported that severe anemic mouse pups and human infants show higher intestinal permeability, bacterial translocation, and both local/systemic inflammation infants.[48, 49] The examined the ileocecal region for altered gene expression. Commendably, the authors have developed their own bioinformatics pipelines to minimize unknown errors in principal component analysis,[50,51] screening of differentially-expressed genes (DEGs),[52,53] hierarchical clustering,[54,55] gene-set enrichment,[56] and quantitative analysis.[57] Comparison of the anemic vs control samples showed 2826 DEGs, with 1041 up- and 1825 downregulated genes. The study has identified genes that need further testing for their role in disruption of the epithelial barrier, local innate immune responses, and inflammation.[48]

Singh et al.[58] have reviewed ethical challenges in neonatal life support. Improved care of premature and criticallyill neonates has dramatically improved survival of these infants. This narrative review examines the application of the four principles of biomedical ethics to contemporary neonatal decision-making,[59] landmark legal cases,[60–62] and persistent controversies in periviability,[63–65] complex congenital anomalies,[40,66] neonatal euthanasia,[67-69] and emerging biotechnologies.[70–72] We need to consider prognostic uncertainty,[73] disability-rights critiques, global disparities, the risks and promises of artificial intelligence in prognostication, and the ongoing prohibition of heritable genome editing.[71,74] Neonatal ethics is a difficult issue because it brings dilemmas between fundamental societal values regarding suffering and the worth of nascent human life.[75,76] There is a need for culturally-sensitive discussion, international collaboration, and development of consensus guidelines.[63]

Guaragni[77] has reviewed advances in viscoelastic coagulation monitoring (VCM).[78] Premature/critically-ill neonates often show clinically-relevant bleeding due to maturational and acquired coagulation disorders. In these patients, VCM has been an exciting advance over the conventional measurements of prothrombin time/International Normalized Ratio and activated partial thromboplastin time.[79] VCM examines whole blood for adequacy of clotting factors, fibrinogen, platelet function, red blood cells, and fibrinolytic processes. In the past few years, several cartridge-based devices have also become available that do not need controlled pipetting. These newer methods are being continuously improved; some instruments maintain core viscoelastic testing (VET) principles of thromboelastography and rotational thromboelastometry[80] but are being advanced with microfluidic cartridges and resonance-based detection, reducing sample handling and operator workload. Another ultrasound-based method measures the stiffness/shear modulus of whole blood as it clots. VET could improve hemostatic management, reduce unnecessary transfusions, and enhance patient outcomes.

Kaushal and Balla[81] are establishing a series of articles that will outline the procedures frequently performed in neonatal intensive care, an attempt to provide continuously updated, much-needed standardized protocols. Here, they have described ultrasound-guided placement of peripherally-inserted central catheters (PICCs) in premature/critically-ill infants.[82–85] Point-of-care ultrasound (POCUS)-guided PICC insertion has improved the first-pass success rates of insertion of PICC lines, reduced complications and reduced radiation exposure.[86] This article outlines an evidence-based, standardized approach to PICC insertion and care in neonates.

Khare et al.[87] reported a case with a congenital pouch colon (CPC) and associated anomalies. These infants show major dilatations in one/more segments of the colon and often show associated anorectal malformations and/or a fistulous communications with the distal urogenital tract, hydronephrosis, hypospadias, bicornuate/septate uterus, absent/double appendix, Meckel's diverticulum, rectal atresia, sacral agenesis, and congenital heart defects. They recently treated a 4-day-old full-term, small-for-gestation female infant weighing 2.3 kg, who was presented with vomiting, abdominal distension, and had been passing stools through one single cloacal opening. Imaging studies and an exploratory laparotomy showed that she had a type-2 CPC with cloaca, a colon-uterine fistula, bicornuate uterus, double appendix, and a Meckel's diverticulum. The fistula was ligated and a pouchostomy was done. The infant stabilized over the next few days and was discharged from the hospital. This case shows that timely identification and management of CPCs can improve the outcome of these patients.

References

1. The Nobel Foundation. Nobel Prize Museum Stockholm, Sweden: Nobel Foundation; 2025 [Available from: https://www.nobelprize.org/about/nobel-museum/.
2. Uppsala-Universitet. Uppsala Universitet Uppsala, Sweden2025 [Available from: https://www.uu.se/en.
3. Global Newborn Society. Inaugural Conference of the Global Newborn Society, Sweden, 2025 New York, USA: Global Newborn Society; 2025 [Available from: https://www.youtube.com/watch?v=KNngtFBJNVM.
4. Regehr G, Varpio L. Conferencing well. Perspect Med Educ. 2022;11(2):101–103. PMID: 35239163. DOI: 10.1007/s40037-022-00704-0.
5. Global Newborn Society. Global Newborn Society Clarksville, Maryland, USA: Global Newborn Society Foundation; 2021 [Available from: https://www.globalnewbornsociety.org/.
6. Asadi A, Marincola FM. A call to reignite the revolutionary spirit of scientific discovery. J Transl Med. 2025;23(1):699. PMID: 40555985. DOI: 10.1186/s12967-025-06669-y.

7. Nelson NM. A decimillennium in neonatology. J Pediatr. 2000;137(5):731–735. PMID: 11060544. DOI: 10.1067/mpd.2000.110422.
8. Santoro D, Zibulsky DA, Roehr CC, Langhammer F, Vento M, Szczapa T, et al. Meeting the need for effective and standardized neonatology training: a pan-European Master's Curriculum. Pediatr Res. 2024;96(5):1195–1200. PMID: 38702380. DOI: 10.1038/s41390-024-03182-8.
9. Calvo F, Karras BT, Phillips R, Kimball AM, Wolf F. Diagnoses, syndromes, and diseases: a knowledge representation problem. AMIA Annu Symp Proc. 2003;2003:802. PMID: 14728307.
10. Wang H, Xiong J, Yang F, Wang W, Yu P, Chen R. Analysis of the global burden of neonatal disorders and risk factors from 1990 to 2021: findings from the global burden of disease study 2021. Front Public Health. 2025;13:1618334. PMID: 41211387. DOI: 10.3389/fpubh.2025.1618334.
11. Costeloe K, Turner MA, Padula MA, Shah PS, Modi N, Soll R, et al. Sharing Data to Accelerate Medicine Development and Improve Neonatal Care: Data Standards and Harmonized Definitions. J Pediatr. 2018;203:437–441 e1. PMID: 30293637. DOI: 10.1016/j.jpeds.2018.07.082.
12. Kay Z. Small Actions, Big Impact: Independently published; 2024.
13. Mummah SA, Robinson TN, King AC, Gardner CD, Sutton S. IDEAS (Integrate, Design, Assess, and Share): A Framework and Toolkit of Strategies for the Development of More Effective Digital Interventions to Change Health Behavior. J Med Internet Res. 2016;18(12):e317. PMID: 27986647. DOI: 10.2196/jmir.5927.
14. Jun K, Lee J. Transformational Leadership and Followers' Innovative Behavior: Roles of Commitment to Change and Organizational Support for Creativity. Behav Sci (Basel). 2023;13(4). PMID: 37102834. DOI: 10.3390/bs13040320.
15. Nobel Prize Museum. Event information and booking conditions. Nobel Prize Museum Stockholm, Sweden: Nobel Prize Museum; 2025 [Available from: https://www.nobelprize.org/uploads/sites/2/2025/06/Booking-information-spring-25.pdf.
16. Nobel Prize Museum. About the Nobel Prize Museum: A small museum with vast content Stockholm, Sweden: Nobel Prize Museum; 2025 [Available from: https://www.nobelprizemuseum.se/en/about-nobel-prize-museum/.
17. Müller-Wille S. Carolus Linnaeus Chicago, USA: Encyclopedia Brittanica; 2025 [Available from: https://www.britannica.com/biography/Carolus-Linnaeus.
18. Britannica-Editors. Anders Celsius Chicago, USA: Encyclopaedia Britannica; 2025 [Available from: https://www.britannica.com/biography/Anders-Celsius.
19. Crawford E. Svante Arrhenius Chicago, USA: Encyclopedia Britannica; 2025 [Available from: https://www.britannica.com/biography/Svante-Arrhenius.
20. Britannica-Editors. Dag Hammarskjöld Chicago, USA: Encyclopedia Britannica; 2025 [Available from: https://www.britannica.com/biography/Dag-Hammarskjold.
21. Raikar SP. Greenwich meridian Chicago, USA: Encyclopedia Britannica; 2025 [Available from: https://www.britannica.com/place/Greenwich-meridian.
22. Roach GD, Sargent C. Interventions to Minimize Jet Lag After Westward and Eastward Flight. Front Physiol. 2019;10:927. PMID: 31417411. DOI: 10.3389/fphys.2019.00927.
23. Alghnimei N, Daghriri Y, Alhwiti A, Alsharari N, Eltayeb A, Alshama D, et al. Screening for Developmental Dysplasia of the Hip in Saudi Arabia: Technically Feasible, but Social Barriers Persist. Newborn (Clarksville, Md). 2025;4(4):159–164. DOI: 10.5005/jp-journals-11002-0140.
24. Nath G. Society For Bacteriophage Research and Therapy Varanasi, India: Society For Bacteriophage Research and Therapy; 2025 [Available from: http://sbrtganga.com/.
25. Gangwar M, Karn S, Chhibber S, Kutter E, Nath G. Editorial: Pharmacological and Immunological Action of Bacteriophages: Focus on Phage Therapy. Front Pharmacol. 2022;13:856542. PMID: 35517796. DOI: 10.3389/fphar.2022.856542.
26. Singh AN, Singh A, Singh SK, Nath G. Klebsiella pneumoniae infections and phage therapy. Indian J Med Microbiol. 2024;52:100736. PMID: 39357832. DOI: 10.1016/j.ijmmb.2024.100736.
27. Patil R, Dehari D, Chaudhuri A, Kumar DN, Kumar D, Singh S, et al. Recent advancements in nanotechnology-based bacteriophage delivery strategies against bacterial ocular infections. Microbiol Res. 2023;273:127413. PMID: 37216845. DOI: 10.1016/j.micres.2023.127413.
28. Bhargava K, Nath G, Bhargava A, Aseri GK, Jain N. Phage therapeutics: from promises to practices and prospectives. Appl Microbiol Biotechnol. 2021;105(24):9047–9067. PMID: 34821965. DOI: 10.1007/s00253-021-11695-z.
29. Bhargava K, Nath G, Dhameja N, Kumar R, Aseri GK, Jain N. Bacteriophage therapy for Escherichia coli-induced urinary tract infection in rats. Future Microbiol. 2023;18:323–334. PMID: 37140267. DOI: 10.2217/fmb-2022-0107.
30. Dehari D, Chaudhuri A, Kumar DN, Patil R, Gangwar M, Rastogi S, et al. A Bacteriophage Microgel Effectively Treats the Multidrug-Resistant Acinetobacter baumannii Bacterial Infections in Burn Wounds. Pharmaceuticals (Basel). 2023;16(7). PMID: 37513854. DOI: 10.3390/ph16070942.
31. Gangwar M, Rastogi S, Singh D, Shukla A, Dhameja N, Kumar D, et al. Immunological and safety profile of bacteriophage therapy: A pre-clinical study. J Appl Microbiol. 2022;133(3):1446–1460. PMID: 35633293. DOI: 10.1111/jam.15642.
32. Archana A, Patel PS, Kumar R, Nath G. Neutralizing antibody response against subcutaneously injected bacteriophages in rabbit model. Virusdisease. 2021;32(1):38–45. PMID: 33969154. DOI: 10.1007/s13337-021-00673-8.
33. Srivastava P, Mishra CP, Nath G. Bacteriophages Can Make a Difference in Water Quality: Evidence From a Community-Based Study From North India. Cureus. 2022;14(8):e27551. PMID: 36059352. DOI: 10.7759/cureus.27551.
34. Teklemariam AD, Al-Hindi RR, Qadri I, Alharbi MG, Ramadan WS, Ayubu J, et al. The Battle between Bacteria and Bacteriophages: A Conundrum to Their Immune System. Antibiotics (Basel). 2023;12(2). PMID: 36830292. DOI: 10.3390/antibiotics12020381.
35. Koskella B, Meaden S. Understanding bacteriophage specificity in natural microbial communities. Viruses. 2013;5(3):806–823. PMID: 23478639. DOI: 10.3390/v5030806.
36. Singh S, Kaushal M, Guaragni B, Aversa S, Alghnimei N, Hayat MR, et al. Systematized Systemic Sonographic Surveillance (S4). Newborn (Clarksville, Md). 2025;4(4):181–193. DOI: 10.5005/jp-journals-11002-0142.
37. More K, Sahni M, Maheshwari A. Systemized Systemic Sono-screening (S4) Protocol: Initial Findings. Newborn (Clarksville, Md). 2025;4(3):153–155. DOI: 10.5005/jp-journals-11002-0135.
38. Siems A, Banks R, Holubkov R, Meert KL, Bauerfeld C, Beyda D, et al. Structured Chart Review: Assessment of a Structured Chart Review Methodology. Hosp Pediatr. 2020;10(1):61–69. PMID: 31879317. DOI: 10.1542/hpeds.2019-0225.
39. Fraleigh CDM, Duff E. Point-of-care ultrasound: An emerging clinical tool to enhance physical assessment. Nurse Pract. 2022;47(8):14–20. PMID: 35877142. DOI: 10.1097/01.NPR.0000841944.00536.b2.

40. Gostin L. A moment in human development: legal protection, ethical standards and social policy on the selective non-treatment of handicapped neonates. Am J Law Med. 1985;11(1):31–78. PMID: 3832944.
41. Suqaty R, Alomran AK, Alkhalifah MK, Aldughaythir SS, Albeshry AM, Aldilaijan Y, et al. How Ready are Pediatricians and Family Physicians in Saudi Arabia to Perform Clinical Screening of Developmental Dysplasia of the Hip? J Multidiscip Healthc. 2023;16:2567–2576. PMID: 37667798. DOI: 10.2147/JMDH.S416459.
42. Prasad CS, Maheshwari A. Maternal Determinants of Neurodiversity in the Developing Brain: An Integrative Review of Biological, Environmental and Psychological Factors. Newborn (Clarksville, Md). 2025;4(4):203–220. DOI: 10.5005/jp-journals-11002-0147.
43. Dawson G, Rieder AD, Johnson MH. A Developmental Social Neuroscience Perspective on Infant Autism Interventions. Annu Rev Dev Psychol. 2023;5:89–113. PMID: 40521253. DOI: 10.1146/annurev-devpsych-120621-042753.
44. Swanepoel A. ADHD and ASD are Normal Biological Variations as part of Human Evolution and are not "Disorders". Clin Neuropsychiatry. 2024;21(6):451–454. PMID: 39839603. DOI: 10.36131/cnfioritieditore20240601.
45. Thompson RA. Early Brain Development and Public Health. Dela J Public Health. 2024;10(4):6–11. PMID: 39493243. DOI: 10.32481/djph.2024.10.03.
46. Hens K, Van Goidsenhoven L. Developmental diversity: Putting the development back into research about developmental conditions. Front Psychiatry. 2022;13:986732. PMID: 36684021. DOI: 10.3389/fpsyt.2022.986732.
47. Das JK, Maheshwari A. A High-Throughput Analysis of Gene Expression in the Intestine in Severely-Anemic Mouse Pups. Newborn (Clarksville, Md). 2025;4(4):165–177. DOI: 10.5005/jp-journals-11002-0145.
48. Maheshwari A. Severe anemia predisposes very premature infants to transfusion-associated necrotizing enterocolitis. Semin Fetal Neonatal Med. 2025;30(1):101615. PMID: 40059009. DOI: 10.1016/j.siny.2025.101615.
49. MohanKumar K, Namachivayam K, Song T, Jake Cha B, Slate A, Hendrickson JE, et al. A murine neonatal model of necrotizing enterocolitis caused by anemia and red blood cell transfusions. Nat Commun. 2019;10(1):3494. PMID: 31375667. DOI: 10.1038/s41467-019-11199-5.
50. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. Philos Trans A Math Phys Eng Sci. 2016;374(2065):20150202. PMID: 26953178. DOI: 10.1098/rsta.2015.0202.
51. Karuppusami R, Antonisamy B, Premkumar PS. Functional principal component analysis for identifying the child growth pattern using longitudinal birth cohort data. BMC Med Res Methodol. 2022;22(1):76. PMID: 35313828. DOI: 10.1186/s12874-022-01566-0.
52. Jiang Z, Luo Y, Wei L, Gu R, Zhang X, Zhou Y, et al. Bioinformatic Analysis and Machine Learning Methods in Neonatal Sepsis: Identification of Biomarkers and Immune Infiltration. Biomedicines. 2023;11(7). PMID: 37509492. DOI: 10.3390/biomedicines11071853.
53. Liu X, Zhang X, Li L, Wang J, Chen Y, Wu L. Bioinformatics analysis of potential key genes and pathways in neonatal necrotizing enterocolitis. BMC Pediatr. 2022;22(1):658. PMID: 36371157. DOI: 10.1186/s12887-022-03721-4.
54. Li H, Hong F. Cluster-Rasch models for microarray gene expression data. Genome Biol. 2001;2(8):RESEARCH0031. PMID: 11532215. DOI: 10.1186/gb-2001-2-8-research0031.
55. Do JH, Choi DK. Clustering approaches to identifying gene expression patterns from DNA microarray data. Mol Cells. 2008;25(2):279-88. PMID: 18414008.
56. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A. 2005;102(43):15545–15550. PMID: 16199517. DOI: 10.1073/pnas.0506580102.
57. Hardin J, Finnell RH, Wong D, Hogan ME, Horovitz J, Shu J, et al. Whole genome microarray analysis, from neonatal blood cards. BMC Genet. 2009;10:38. PMID: 19624846. DOI: 10.1186/1471-2156-10-38.
58. Singh S, Buddington SS, Huseynova R. Ethical Challenges in Neonatal Life Support. Newborn (Clarksville, Md). 2025;4(4):194–202. DOI: 10.5005/jp-journals-11002-0144.
59. Varkey B. Principles of Clinical Ethics and Their Application to Practice. Med Princ Pract. 2021;30(1):17–28. PMID: 32498071. DOI: 10.1159/000509119.
60. Baker AC, Mercurio MR, Donn SM, Fanaroff JM. Ethical and Legal Perspectives on the Treatment of Hypoxic Ischemic Encephalopathy in the Newborn. Clin Perinatol. 2024;51(3):725–734. PMID: 39095106. DOI: 10.1016/j.clp.2024.04.010.
61. Carter BS. Neonatologists and bioethics after Baby Doe. J Perinatol. 1993;13(2):144–150. PMID: 8515309.
62. Moreno JD. Ethical and legal issues in the care of the impaired newborn. Clin Perinatol. 1987;14(2):345–360. PMID: 3595056.
63. Kornhauser Cerar L, Lucovnik M. Ethical Dilemmas in Neonatal Care at the Limit of Viability. Children (Basel). 2023;10(5). PMID: 37238331. DOI: 10.3390/children10050784.
64. Arimitsu T, Hatayama K, Gaughwin K, Kusuda S. Ethical considerations regarding the treatment of extremely preterm infants at the limit of viability: a comprehensive review. Eur J Pediatr. 2025;184(2):140. PMID: 39814940. DOI: 10.1007/s00431-025-05976-2.
65. Berger TM, Bernet V, El Alama S, Fauchere JC, Hosli I, Irion O, et al. Perinatal care at the limit of viability between 22 and 26 completed weeks of gestation in Switzerland. 2011 revision of the Swiss recommendations. Swiss Med Wkly. 2011;141:w13280. PMID: 22009720. DOI: 10.4414/smw.2011.13280.
66. Foo KB. Medico-legal and ethical problems associated with treatment of children born with congenital malformations. Singapore Med J. 1994;35(2):184–189. PMID: 7524162.
67. Baral VR, Lim Y, Edison P, Menikoff JA. Ethical Dilemmas in Newborn Infants with Hypoxic Ischemic Encephalopathy. Asian Bioeth Rev. 2025;17(2):237–249. PMID: 40225795. DOI: 10.1007/s41649-024-00337-x.
68. Soltani Gerdfaramarzi M, Bazmi S. Neonatal end-of-life decisions and ethical perspectives. J Med Ethics Hist Med. 2020;13:19. PMID: 33552452. DOI: 10.18502/jmehm.v13i19.4827.
69. Vanden Eijnden S, Martinovici D. Neonatal euthanasia: A claim for an immoral law. Clin Ethics. 2013;8(2-3):75–84. PMID: 24068880. DOI: 10.1177/1477750913499494.
70. Cummings CL, Mercurio MR. Ethics of emerging technologies and their transition to accepted practice: intestinal transplant for short bowel syndrome. J Perinatol. 2012;32(10):752–756. PMID: 23014383. DOI: 10.1038/jp.2012.69.
71. Lantos JD. Neonatal bioethics, AI, and genomics. Early Hum Dev. 2024;198:106130. PMID: 39405800. DOI: 10.1016/j.earlhumdev.2024.106130.
72. Kearney E, Wojcik A, Babu D. Artificial intelligence in genetic services delivery: Utopia or apocalypse? J Genet Couns. 2020;29(1):8–17. PMID: 31749317. DOI: 10.1002/jgc4.1192.

73. Krick JA, Weiss EM, Snyder A, Haldar S, Campelia GD, Opel DJ. Living with the Unknown: A Qualitative Study of Parental Experience of Prognostic Uncertainty in the Neonatal Intensive Care Unit. Am J Perinatol. 2021;38(8):821–827. PMID: 31899927. DOI: 10.1055/s-0039-3402722.
74. Pougnet R, Derbez B, Troadec MB. Mapping the 'Ethical' Controversy of Human Heritable Genome Editing: a Multidisciplinary Approach. Asian Bioeth Rev. 2023;15(2):189–204. PMID: 37035482. DOI: 10.1007/s41649-022-00234-1.
75. Pasaron R. Neonatal bioethical perspectives: practice considerations. Neonatal Netw. 2013;32(3):184–192. PMID: 23666188. DOI: 10.1891/0730-0832.32.3.184.
76. Catlin A, Armigo C, Volat D, Vale E, Hadley MA, Gong W, et al. Conscientious objection: a potential neonatal nursing response to care orders that cause suffering at the end of life? Study of a concept. Neonatal Netw. 2008;27(2):101-108. PMID: 18431964. DOI: 10.1891/0730-0832.27.2.101.
77. Guaragni B. Advances in Viscoelastic Coagulation Monitoring. Newborn (Clarksville, Md). 2025;4(4):225–227. DOI: 10.5005/jp-journals-11002-0139.
78. Amelio GS, Raffaeli G, Amodeo I, Gulden S, Cortesi V, Manzoni F, et al. Hemostatic Evaluation With Viscoelastic Coagulation Monitor: A Nicu Experience. Front Pediatr. 2022;10:910646. PMID: 35620150. DOI: 10.3389/fped.2022.910646.
79. Patil P, Sehgal T, Goswami P, Gaur M, Khan M, Pandey S, et al. Assessment of Stability of Prothrombin Time, International Normalized Ratio, and Activated Partial Thromboplastin Time Under Different Storage Conditions in Human Plasma. Cureus. 2022;14(1):e21268. PMID: 35178322. DOI: 10.7759/cureus.21268.
80. Hartmann J, Hermelin D, Levy JH. Viscoelastic testing: an illustrated review of technology and clinical applications. Res Pract Thromb Haemost. 2023;7(1):100031. PMID: 36760779. DOI: 10.1016/j.rpth.2022.100031.
81. Kaushal M, Balla KC. Clinical Procedures: Use of Peripherally Inserted Central Catheters in Neonates. Newborn (Clarksville, Md). 2025;4(4):178–180. DOI: 10.5005/jp-journals-11002-0143.
82. Ozkiraz S, Gokmen Z, Anuk Ince D, Akcan AB, Kilicdag H, Ozel D, et al. Peripherally inserted central venous catheters in critically ill premature neonates. J Vasc Access. 2013;14(4):320–324. PMID: 23817952. DOI: 10.5301/jva.5000157.
83. Gomes de Souza NM, Silveira Rocha R, Pinheiro Ferreira R, Bastos da Silveira Reis C, Souza Bandeira RS, Facanha Melo AP. Comparing the use of silicone and polyurethane Peripherally Inserted Central Catheters in newborns: A retrospective study. J Clin Nurs. 2021;30(23–24):3439–3447. PMID: 34545654. DOI: 10.1111/jocn.15799.
84. Wrightson DD. Peripherally inserted central catheter complications in neonates with upper versus lower extremity insertion sites. Adv Neonatal Care. 2013;13(3):198–204. PMID: 23722492. DOI: 10.1097/ANC.0b013e31827e1d01.
85. Luister A, Khostwal N, Deindl P, Herrmann J, Singer D, Ebenebe CU. Recommendations for Peripherally Inserted Central Catheter Insertion Depths in Neonates. Neonatology. 2023;120(2):263–267. PMID: 36596282. DOI: 10.1159/000528076.
86. Lin SY, Chiang MC, Wu WH, Wu IH, Lai MY, Chu SM, et al. Point-of-care ultrasound (POCUS) for tip localization of neonatal peripherally inserted central catheter (PICC): A prospective study. Pediatr Neonatol. 2024;65(4):375–380. PMID: 38114415. DOI: 10.1016/j.pedneo.2023.07.008.
87. Khare AK, Baindur AJ, Tanger RC. Case report: A Congenital Pouch Colon with Anorectal Malformation and Associated Anomalies. Newborn (Clarksville, Md). 2025;4(4):221–224. DOI: 10.5005/jp-journals-11002-0146.

ORIGINAL RESEARCH

Screening for Developmental Dysplasia of the Hip in Saudi Arabia: Technically Feasible, but Social Barriers Persist

Naief Alghnimei[1], Yaquob Daghriri[2], Attallah Alhwiti[3], Nayef Alsharari[4,5,19,20], Ahmed Eltayeb[6], Duha Alshama[7], Mohammed Gharib[8], Malak Almehmadi[9], Yousef Alshammari[10], Saif Alanazi[11], Meshari Alhawiti[12], Abdulrahman Alelyani[13], Abdullah Alayda[14], Zaben Alanazi[15], Ali Daghriri[16], Yahya Ethawi[17–20]

Received on: 26 October 2025; *Accepted on*: 03 December 2025; *Published on*: 15 January 2026

Abstract

Objectives: Developmental dysplasia of hip (DDH) is a complex spectrum of hip abnormalities, where timely diagnosis and management can improve outcomes. Evaluation soon after birth and during early infancy can help identify at-risk infants. In this study, we present a brief review of our DDH screening program and a retrospective observational study of the barriers to DDH screening/follow-up in a large clinical cohort of infants in the Kingdom of Saudi Arabia (KSA).

Methods: This retrospective observational cohort study was conducted in the KSA for an 8-month period between December 2023 and July 2024. We evaluated infants with one risk factor and/or positive clinical exam for DDH and followed them with at least one in-person visit, followed by direct clinical and/or telephone contact. A standardized questionnaire was used to understand parental perception and factors affecting compliance with the screening process.

Results: We followed a cohort of 3,598 infants; 95 were identified as high-risk for DDH. The most frequent prenatal risk factors were breech deliveries, positive family history, preterm birth, and twin gestation. In the DDH follow-up program, the overall compliance rate was 47/95 (49.5%). The major reason for missing the other 48 (50.5%) at-risk patients was ineffective communication. The contact information had not been recorded correctly in 36 of 48 cases (75%). In 11 (23%), parents were not aware of the importance of timely diagnosis and close follow-up. In 13 (27%), the primary difficulty was access to medical support because of long distance/difficult terrain. Some families either did not believe in the need for screening [2/48 (4.2%)] or missed their appointment [4/48 (8.3%)]. In some cases, medical responsibilities related to other members in the household and cultural beliefs about DDH screening may have contributed to noncompliance. Improvement in parental education regarding DDH raised the compliance rate with follow-up by 20%.

Conclusion: Parental noncompliance with DDH follow-up screening programs is influenced by multiple factors. The most frequent reasons were related to difficulties with communication and inaccuracy of parental contact information. Parental education about the importance of timely diagnosis of DDH and the risks of delayed intervention is essential. The geographic location of the families from medical facilities was also important; the access through long distances and/or difficult terrain needs to be improved.

Keywords: Barriers, Education, Healthcare access, Hip displacement, Hip dislocation, Neonates, Parental compliance, Screening programs, Telemedicine.

Newborn (2025): 10.5005/jp-journals-11002-0140

Key Points

- Developmental dysplasia of the hip (DDH) is a complex spectrum of hip abnormalities, many of which can be managed following timely diagnosis and prompt management.
- The authors evaluated 3,598 infants for their ante-, peri-, and postnatal risk factors. In this cohort, 95 were identified to be at high-risk, and DDH was confirmed in 48.
- The most frequent prenatal risk factors were breech deliveries, positive family history, preterm birth, and twin gestation.
- Timely management of DDH was difficult because of (i) inaccurate contact information of families; (ii) lack of parental awareness; and (iii) difficulties related to distance/terrain between the families and healthcare facilities.
- We need to promote parental education and improve access to screening clinics.

Introduction

Developmental dysplasia of the hip is a spectrum of complex hip disorders seen in neonates, ranging from stable acetabular

[1,2,6]Department of Orthopedic Surgery, King Salman Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia

[3,7,8]Department of Neonatology, King Salman Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia

[4]Department of Neonatology, Children and Women's Hospital, Tabuk, Kingdom of Saudi Arabia

[5]My Child Medical Charity Association, Tabuk, Kingdom of Saudi Arabia

[9–11]Department of Orthopedics, King Salman Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia

[12–14]Department of Medicine, Faculty of Medicine and Surgery, University of Tabuk, Tabuk, Kingdom of Saudi Arabia

[15,16]Department of Radiology, King Salman Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia

[17]Department of Neonatology, Tabuk Children and Women Hospital, Tabuk, Kingdom of Saudi Arabia

[18]Neonatal Transport Program, Tabuk, Kingdom of Saudi Arabia

[19]Mychild Organization, Tabuk, Kingdom of Saudi Arabia

[20]Neonatology Unit, Maternal and Children Hospital in Tabuk, Kingdom of Saudi Arabia

dysplasia to hip subluxation and dislocation.[1–3] These conditions are often asymptomatic during the first year after birth and can go unnoticed until the infant begins to stand or walk. Early detection and treatment of DDH can improve outcomes; however, delayed diagnosis often increases the need for surgical procedures and leads to suboptimal outcomes. If left untreated, DDH may cause significant pain, early osteoarthritis, and the need for hip replacement as early as the fourth decade of life.[1–3] This affects not only individual patients but also their families and healthcare systems.[4]

The incidence of DDH has been recorded as 2.4–6.7 per 1,000 newborns in Europe and the United States.[5–9] In the Kingdom of Saudi Arabia (KSA), one study recorded an incidence of 3.5 per 1,000 births, with most cases diagnosed after the initiation of walking because of inadequate screening programs.[8,10] A systematic review (1980–2018) showed the average prevalence as 10.5 per 1,000 newborns.[8] Consanguinity, genetic factors, traditional swaddling, and some environmental factors were associated with increased risk.[8,11,12] Nationwide data are currently being compiled to guide targeted prevention efforts. There is a need for clearly articulated, standardized guidelines for clinical screening for DDH.[13,14]

Existing studies have identified a higher risk of DDH in first-born infants of female gender, those with a positive family history, tight lower extremity swaddling, and breech presentation at ≥34 weeks of gestation. The mode of delivery or external cephalic versions did not increase the risk.[15] Infants with torticollis, plagiocephaly, metatarsus adductus, calcaneovalgus deformity, or those born following oligohydramnios, multiple-gestation pregnancy, or with a birth weight >4 kg were also at higher risk.[16] Breech presentation appears to be a major risk factor, with the incidence of DDH as high as 27%.[16,17]

Hip examination can help in detection of DDH. Asymmetric skin creases, a limited hip abduction, and a positive Ortolani or Barlow's maneuver in the first 3 months after birth should trigger further evaluation.[18,19] The need for universal screening for DDH is not questioned, but the specific steps still need to be defined.[20] The American Academy of Pediatrics (AAP) and the DDH Task Force of Canada emphasize focused clinical screening mainly using the Ortolani test. Universal ultrasonographic screening is not recommended yet.[21,22] In the United Kingdom, the Newborn and Infant Physical Examination (NIPE) handbook recommends serial steps for evaluation at 6–8 weeks after birth.[5,23,24]

In infants with risk factors or suggestive clinical findings at the age of 6 weeks after birth, sonographic examination can help. The AAP suggests that most of the minor ultrasound abnormalities seen at 6–16 weeks after birth will resolve. However, in Germany, universal sonographic screening has helped reduce surgical intervention by 80%.[24] Universal physical examinations and ultrasounds have also improved the outcomes in Hong Kong.[25] The total pooled incidence estimates for early detected DDH were 23 per 1,000 newborns among those with universal ultrasonographic screening, 4.4 per 1,000 among those with selective ultrasonographic screening, and 8.4 per 1,000 newborns with clinical screening.[26]

Overall, clinical neonatal screening using the Ortolani and Barlow maneuvers has shown an incidence of late-presenting DDH of 0.37/1,000 live births.[27–29] The average sensitivity was 60% (range 60–85%), and the specificity was 96% (range 90–98%). In combination with sonographic screening of all infants, the incidence was lower at 0.13/1,000 live births, with a sensitivity of 74% (88–95%) and specificity of 99.3% (range 95–98%). A combination of clinical screening followed by selective ultrasound showed an incidence of 0.53/1,000 live births, with a sensitivity of 82% (range 75–90%) and specificity of 97% (range 95–98%). These data show considerable variability in sensitivity and specificity based on the experience of the examiner(s), the timing of the screening, and the criteria used to define a positive screening result. Compared to universal ultrasound screening, selective ultrasound screening in clinically chosen infants may miss a few cases of DDH but it might be more cost-effective in resource-limited settings. In this study, we report the current stage of our efforts in KSA to develop a DDH screening program.

Corresponding Author: Naief Alghnimei, Department of Orthopedic Surgery, King Salman Armed Forces Hospital, Tabuk, Kingdom of Saudi Arabia, Phone: +966 540212188, e-mail: naief.alghnimei@gmail.com

How to cite this article: Alghnimei N, Daghriri Y, Alhwiti A, *et al*. Screening for Developmental Dysplasia of the Hip in Saudi Arabia: Technically Feasible, but Social Barriers Persist. Newborn 2025;4(4): 159–164.

Source of support: Nil

Conflict of interest: Dr Yahya Ethawi is associated as the Editorial Board Member of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of the Editorial Board Member and his research group.

Methods

We conducted a retrospective cohort study in the Tabuk province of the KSA using chart review and recall of parents during the period October 2023–November 2024. The goal was to determine the incidence and compliance with the DDH screening program. We reviewed the charts of all infants with:

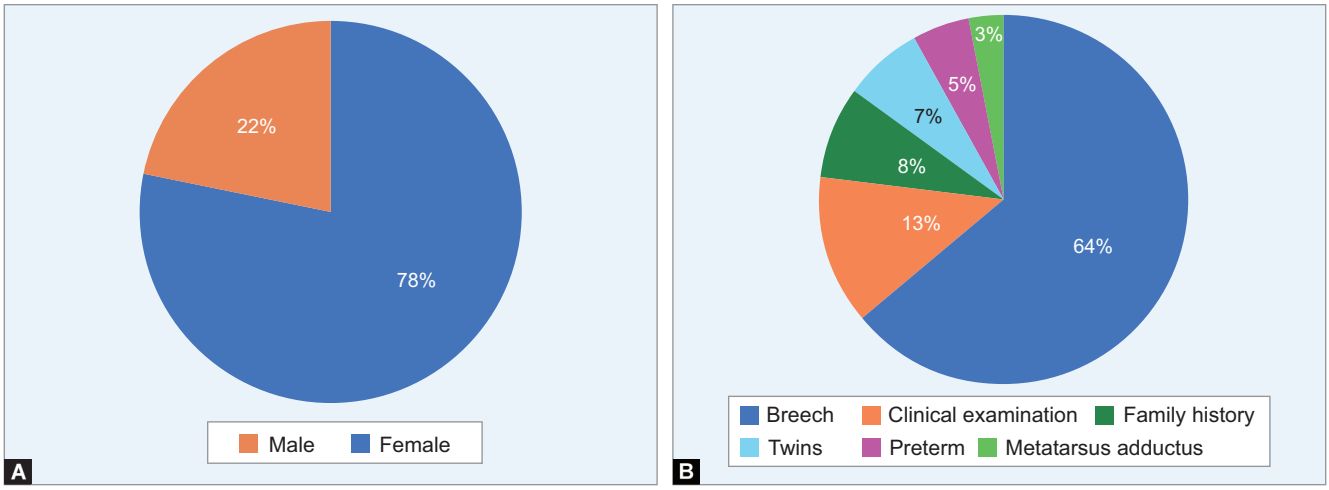
- Previous family history of DDH,
- Breech presentations,
- Positive physical examination as with the Ortolani and Barlow tests,
- Foot deformities,
- Oligohydramnios, and
- Torticollis.

Our cohort showed the following associations with DDH (Fig. 1).

Patients with multisystem syndromes were excluded. We recorded patient demographics, reasons for referral, risk factors for DDH, and physical findings suggestive of DDH. In all cases, the clinical evaluation were performed by healthcare providers who had been trained to identify at-risk infants and follow the DDH risk pathway (Fig. 2).

We have standardized the clinical examination of the hip joint. The infant is placed supine on a flat, warm surface in a quiet environment and then examined using standardized Ortolani and Barlow maneuvers.[28] For the Ortolani test, the hips are flexed at 90 degrees, and the clinician positions the index and middle fingers on the lateral side of the greater trochanter and the thumb medially along the groin crease. The flexed and adducted hip is abducted while applying gentle traction and pressure on the greater trochanter. A dislocated hip relocates with a "clunk". In the Barlow test, the knee is gently adducted. A downward force is then applied along the femoral axis to identify any posterior subluxation or dislocation through a palpable sensation.

Ultrasound is the preferred imaging method for assessing DDH in newborns, especially before the femoral head begins to ossify around 4–6 months of age. Using high-frequency linear transducers, the examination evaluates the shape and depth of the acetabulum, the position and stability of the femoral head, and the



Figs 1A and B: Risk factors associated with DDH in our cohort

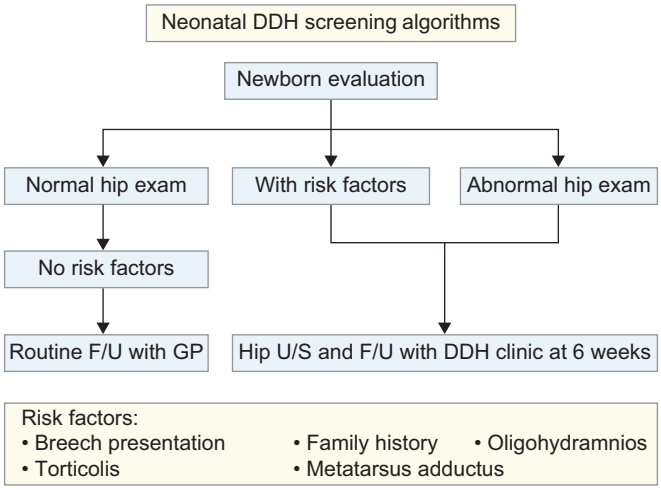


Fig. 2: Developmental dysplasia of hip screening in our clinics

F/U, follow-up; GP, general practitioner; U/S, ultrasound

relationship of cartilaginous structures that are not visible on X-ray. The Graf technique is most commonly used, providing standardized coronal images that allow measurement of the alpha and beta angles to classify hips as normal, immature, dysplastic, subluxated, or dislocated.[30] Ultrasound is typically performed at 4–6 weeks of age in infants with risk factors such as breech presentation, a positive family history, or an abnormal physical exam, though clearly abnormal findings may be assessed earlier. This noninvasive, radiation-free modality supports early detection and appropriate intervention to prevent long-term complications.

Each at-risk patient was then examined using ultrasound imaging of both hip joints using HD 11 and EPIC machines. The neonate was positioned on her/his side (coronal flexion view) for real-time scanning using the modified Harcke dynamic and modified Graf static methods (Fig. 2).[31] The alpha (α) angle is measured between the iliac line and the bony acetabular roof; normal values are >60°. In DDH, the α-angle may be smaller and indicates a shallow acetabulum. The beta (β) angle, measured between the iliac line and the cartilaginous acetabular labrum, should be <55°.[32] Larger β-angles indicate displacement of labrum and instability. In most infants, >50% of the femoral head is covered by the acetabulum, and coverage <50% suggests dysplasia or subluxation.

Dynamic ultrasound can show findings about hip stability.[31] Stress maneuvers can show subluxation, dislocation, delayed/abnormal reduction, or a loose hip with excessive movement.[33] Finally, the femoral head can show lateral and/or posterior displacement, or it could appear high-riding in dislocation (Figs 3 and 4).

The α-angle represents the osseous coverage of the femoral head and is measured between a baseline horizontal line across the ilium and the bony roof; normal measurements are >60°. The β-angle measures the cartilaginous roof and is measured between the baseline and the cartilaginous roof line, should be <55°.

The Graf Classification (Simplified) views DDH in the following categories:[34]

Type	*Alpha angle*	*Interpretation*
I	≥60°	Normal hip
IIa	50–59° (infants <3 months)	Immature hip (may normalize spontaneously)
IIb	50–59° (≥3 months)	Dysplastic
III	<50°	Subluxated hip
IV	Severely abnormal	Dislocated hip

Our DDH management protocol recommends the application of a Pavlik harness in all timely diagnosed infants.[35] This is a well-standardized dynamic flexion–abduction orthosis used to treat infants with DDH up to 6 months of age. There are two shoulder straps that maintain a chest strap at the nipple line, and two stirrups on the lower extremities. Each stirrup has an anteromedial flexion strap and a posterolateral abduction strap; these typically achieve hip stability within 4 weeks. However, the harness is kept in place until both the clinical examination and hip X-rays (Fig. 5) have normalized. Hip joint reduction was evaluated using weekly hip ultrasound assessments for 3 weeks, followed by an X-ray at 6 weeks. In all seven cases, the hip was successfully reduced with no signs of dislocation.

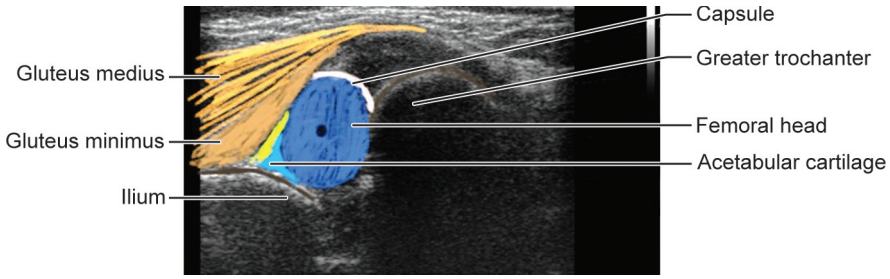
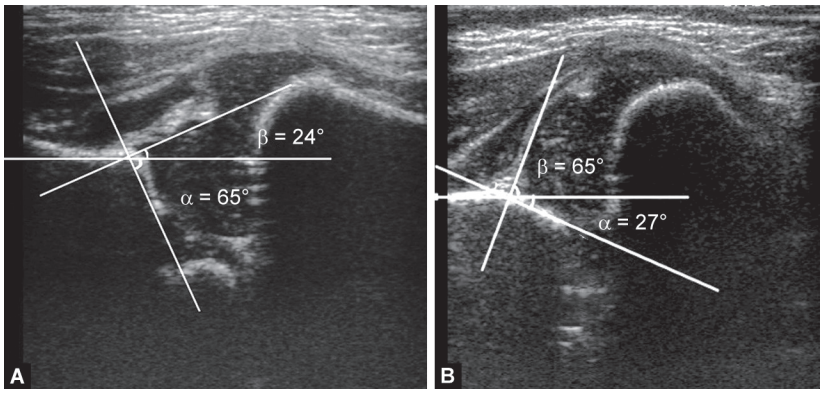


Fig. 3: Key structures seen on sonography of hip joints



Figs 4A and B: Sonographic measurements of the hip joint. (A) Normal hip joint; and (B) Developmental dysplasia of hip

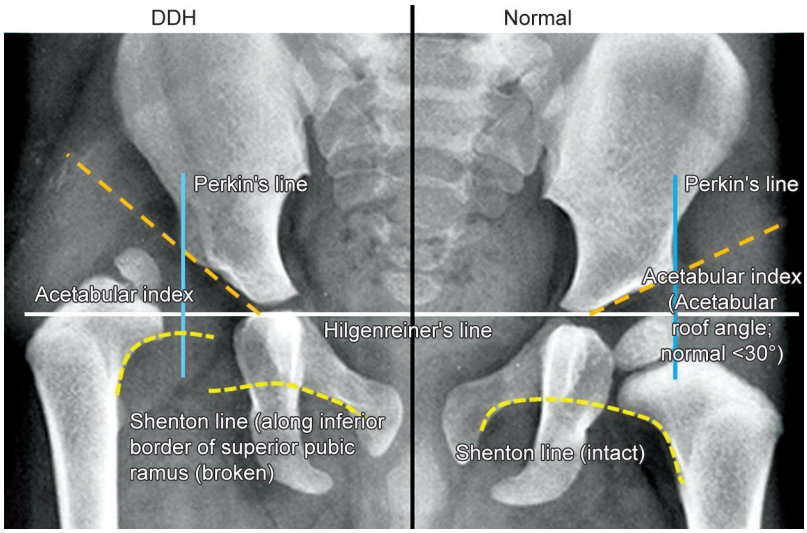


Fig. 5: X-ray of a young infant with DDH. The image lines are as described in the adjoining text in the article

Radiological Assessment

Radiographs are less useful in newborns because the femoral head ossification center appears at 4–6 months.[36] However, some signs may still be seen. In an anteroposterior view of the pelvis, several lines are drawn as reference guides for normal structural relationships of the hip joints (Fig. 5). Hilgenreiner's line is a horizontal line passing through the inferior aspect of both triradiate cartilages; it serves as a baseline for other measurements.[37] Normally, the femoral head ossification should be inferior to this line. The acetabular index (AI) is the angle formed by Hilgenreiner's line and a line from a point on the lateral triradiate cartilage to a point on lateral margin of acetabulum.[3,38] The normal value in neonates is <30°; higher AIs may suggest acetabular dysplasia.

Perkin's line is drawn perpendicular to Hilgenreiner's line, passing through the most lateral point of the acetabular roof.[37] In a normal hip, the upper femoral epiphysis is located in the inferomedial quadrant, meaning it is below Hilgenreiner's line and medial to Perkin's line. Lateral displacement of the femoral head is an indicator of DDH. A Shenton's line can be plotted as a

Table 1: Reasons for nonadherence to DDH screening and management programs

Cause of nonadherence	*Number*	*Percentage*
Difficulties with communication	18	37.5
Not well informed or not clear about the seriousness of the condition	11	22.9
Difficulty in accessing healthcare facilities	13	27
Missed appointment	4	8.5
Preoccupied/lack of parental trust	2	4.1
Total	48	100

smooth arc along the inferior border of femoral neck to superior pubic ramus. It can appear broken in subluxation/dislocation.[39] Finally, the ossification nucleus may be small or absent in DDH in young infants.

Results

We reviewed the medical records of 3598 infants in this study. The overall compliance rate in the DDH follow-up programs was 47/95 (49.5%). The primary reasons for non-adherence with the screening process were related to difficulties in communication (Table 1). The correct contact information was not available in 18/48 (37.5%). Other reasons included paucity of information about the seriousness of the condition [11/48 (22.9%)], difficulties in reaching the healthcare centers from remote areas [13/48 (27%)], lack of parental trust in the programs [2/48 (4.1%)], and missed appointments [4/48 (8.2%)]. Concomitant family responsibilities and cultural beliefs might have also led to the non-compliance in some cases. Encouragingly, parental education improved the rates of DDH screening by 20% (11/48, 22.9%).

After taking the subjects' loss into consideration, the overall incidence of DDH can be projected to be about 9/(3,598 × 49.5) = 5/1000 births in our region. The most frequent risk factors were breech delivery [61/95 (64%)], positive family history [8/95 (8%)], prematurity [5/95 (5%)], and twin gestation [7/95 (7%)].

Conclusion

Parental noncompliance with follow-up screening clinics for DDH in our region is concerning and has resulted in delayed diagnosis and treatment in nearly half of all high-risk newborns. This lack of adherence is influenced by several factors, which can be mitigated through key interventions such as establishing a nationwide DDH screening program with universal history and physical examination, along with selective ultrasonography as a standard of care in all neonatology units across the kingdom. We need to improve communication between healthcare providers and parents, with a goal to integrate screening in the mandatory check-ups during the 1st postnatal month. We also need to improve access to screening clinics, enhance training for personnel in primary care clinics, foster community education, and develop awareness campaigns. Engagement through social media can help. Early treatment can improve long-term outcomes for infants with DDH.

Orcid

Yahya Ethawi https://orcid.org/0000-0002-2462-258X

References

1. Burkhart RJ, McNassor R, Acuna AJ, et al. Is prematurity a risk factor for developmental dysplasia of the hip? A systematic review and meta-analysis. J Pediatr Orthop B 2023;32(4):305–311. DOI: 10.1097/BPB.0000000000001021.
2. Dezateux C, Rosendahl K. Developmental dysplasia of the hip. Lancet 2007;369(9572):1541–1552. DOI: 10.1016/S0140-6736(07)60710-7.
3. Nandhagopal T, Tiwari V, De Cicco FL. Developmental Dysplasia of the Hip. StatPearls. Treasure Island (FL); 2025.
4. Shaw BA, Segal LS; Section on Orthopaedics. Evaluation and referral for developmental dysplasia of the hip in Infants. Pediatrics 2016;138(6). DOI: 10.1542/peds.2016-3107.
5. Kolb A, Schweiger N, Mailath-Pokorny M, et al. Low incidence of early developmental dysplasia of the hip in universal ultrasonographic screening of newborns: Analysis and evaluation of risk factors. Int Orthop 2016;40(1):123–127. DOI: 10.1007/s00264-015-2799-2.
6. Loder RT, Skopelja EN. The epidemiology and demographics of hip dysplasia. ISRN Orthop 2011;2011:238607. DOI: 10.5402/2011/238607.
7. Pollet V, Percy V, Prior HJ. Relative risk and incidence for developmental dysplasia of the hip. J Pediatr 2017;181:202–207. DOI: 10.1016/j.jpeds.2016.10.017.
8. Sadat-Aliorcid M. Developmental dysplasia of the hip (DDH) in Saudi Arabia: Time to wake up. A systematic review (1980–2018). Open J Epidemiol 2020;10(2):125–131. DOI: 10.4236/ojepi.2020.102011.
9. Woodacre T, Ball T, Cox P. Epidemiology of developmental dysplasia of the hip within the UK: Refining the risk factors. J Child Orthop 2016;10(6):633–642. DOI: 10.1007/s11832-016-0798-5.
10. Mirdad T. Incidence and pattern of congenital dislocation of the hip in Aseer region of Saudi Arabia. West Afr J Med 2002;21(3):218–222. DOI: 10.4314/wajm.v21i3.28034.
11. Al-Mohrej O, Alsarhani W, Al-Ayedh NK, et al. Characteristics of developmental dysplasia of the hip at a tertiary hospital in Riyadh, Saudi Arabia. J Health Special 2017;5(2):87. DOI: 10.4103/2468-6360.205076.
12. Moraleda L, Albinana J, Salcedo M, et al. Dysplasia in the development of the hip. Rev Esp Cir Ortop Traumatol 2013;57(1):67–77. DOI: 10.1016/j.recot.2012.10.005.
13. Lambeek AF, De Hundt M, Vlemmix F, et al. Risk of developmental dysplasia of the hip in breech presentation: The effect of successful external cephalic version. BJOG 2013;120(5):607–612. DOI: 10.1111/1471-0528.12013.
14. Suqaty R, Alomran AK, Alkhalifah MK, et al. How ready are pediatricians and family physicians in Saudi Arabia to perform clinical screening of developmental dysplasia of the hip? J Multidiscip Healthc 2023;16:2567–2576. DOI: 10.2147/JMDH.S416459.
15. Barr LV, Rehm A. Should all twins and multiple births undergo ultrasound examination for developmental dysplasia of the hip?: A retrospective study of 990 multiple births. Bone Joint J 2013;95-B(1):132–134. DOI: 10.1302/0301-620X.95B1.29927.
16. Bache CE, Clegg J, Herron M. Risk factors for developmental dysplasia of the hip: ultrasonographic findings in the neonatal period. J Pediatr Orthop B 2002;11(3):212–218. DOI: 10.1097/00009957-200207000-00004.
17. Davies R, Talbot C, Paton R. Evaluation of primary care 6- to 8-week hip check for diagnosis of developmental dysplasia of the hip: A 15-year observational cohort study. Br J Gen Pract 2020;70(693):e230–e235. DOI: 10.3399/bjgp20X708269.
18. Laskaratou ED, Eleftheriades A, Sperelakis I, et al. Epidemiology and screening of developmental dysplasia of the hp in Europe: A scoping review. Reports (MDPI) 2024;7(1). DOI: 10.3390/reports7010010.
19. Pavone V, de Cristo C, Vescio A, et al. Dynamic and static splinting for treatment of developmental dysplasia of the hip: A systematic review. Children (Basel) 2021;8(2). DOI: 10.3390/children8020104.
20. Rogers BA, Garbedian S, Kuchinad RA, et al. Total hip arthroplasty for adult hip dysplasia. J Bone Joint Surg Am 2012;94(19):1809–1821. DOI: 10.2106/JBJS.K.00779.
21. Patel H, Canadian Task Force on Preventive Health C. Preventive health care, 2001 update: Screening and management of developmental dysplasia of the hip in newborns. CMAJ 2001;164(12):1669–1677. PMID: 11450209.

22. Weinstein SL, Mubarak SJ, Wenger DR. Developmental hip dysplasia and dislocation: Part II. Instr Course Lect 2004;53:531–542. PMID: 15116642.
23. Kolb A, Chiari C, Schreiner M, et al. Development of an electronic navigation system for elimination of examiner-dependent factors in the ultrasound screening for developmental dysplasia of the hip in newborns. Sci Rep 2020;10(1):16407. DOI: 10.1038/s41598-020-73536-9.
24. von Kries R, Ihme N, Oberle D, et al. Effect of ultrasound screening on the rate of first operative procedures for developmental hip dysplasia in Germany. Lancet 2003;362(9399):1883–1887. DOI: 10.1016/S0140-6736(03)14957-4.
25. Tong SH, Eid MA, Chow W, et al. Screening for developmental dysplasia of the hip in Hong Kong. J Orthop Surg (Hong Kong) 2011;19(2):200–203. DOI: 10.1177/230949901101900214.
26. Kuitunen I, Uimonen MM, Haapanen M, et al. Incidence of neonatal developmental dysplasia of the hip and late detection rates based on screening strategy: A systematic review and meta-analysis. JAMA Netw Open 2022;5(8):e2227638. DOI: 10.1001/jamanetworkopen.2022.27638.
27. Pandey RA, Johari AN. Screening of newborns and infants for developmental dysplasia of the hip: A systematic review. Indian J Orthop 2021;55(6):1388–1401. DOI: 10.1007/s43465-021-00409-2.
28. Paton RW. Screening in developmental dysplasia of the hip (DDH). Surgeon 2017;15(5):290–296. DOI: 10.1016/j.surge.2017.05.002.
29. Shorter D, Hong T, Osborn DA. Screening programmes for developmental dysplasia of the hip in newborn infants. Sao Paulo Med J 2013;1(2):139–140. DOI: 10.1590/S1516-31802013000100028.
30. Jacobino BCP, Galvao MD, da Silva AF, et al. Using the Graf method of ultrasound examination to classify hip dysplasia in neonates. Autops Case Rep 2012;2(2):5–10. DOI: 10.4322/acr.2012.018.
31. Yousefi MR, Yazdanprast M, Neshati H, et al. Comparison static and dynamic ultrasound techniques of DDH: The role of the patient's position. Arch Bone Jt Surg 2024;12(3):191–197. DOI: 10.22038/ABJS.2023.69347.3264.
32. Copuroglu C, Ozcan M, Aykac B, et al. Reliability of ultrasonographic measurements in suspected patients of developmental dysplasia of the hip and correlation with the acetabular index. Indian J Orthop 2011;45(6):553–557. DOI: 10.4103/0019-5413.87131.
33. Kilsdonk I, Witbreuk M, Van Der Woude HJ. Ultrasound of the neonatal hip as a screening tool for DDH: How to screen and differences in screening programs between European countries. J Ultrason 2021;21(85):e147–e153. DOI: 10.15557/JoU.2021.0024.
34. Gulati V, Eseonu K, Sayani J, et al. Developmental dysplasia of the hip in the newborn: A systematic review. World J Orthop 2013;4(2):32–41. DOI: 10.5312/wjo.v4.i2.32.
35. Gahleitner M, Pisecky L, Gotterbarm T, et al. Long-term results of developmental hip dysplasia under therapy with Pavlik Harness. J Pediatr Orthop 2024;44(3):135–140. DOI: 10.1097/BPO.0000000000002575.
36. Kotlarsky P, Haber R, Bialik V, et al. Developmental dysplasia of the hip: What has changed in the last 20 years? World J Orthop 2015;6(11):886–901. DOI: 10.5312/wjo.v6.i11.886.
37. Narayanan U, Mulpuri K, Sankar WN, et al. Reliability of a new radiographic classification for developmental dysplasia of the hip. J Pediatr Orthop 2015;35(5):478–484. DOI: 10.1097/BPO.0000000000000318.
38. Sherman B, Lalonde FD, Schlechter JA. Measuring the acetabular index: An accurate and reliable alternative method of measurement. AJR Am J Roentgenol 2021;217(1):172–176. DOI: 10.2214/AJR.20.23358.
39. Rhee PC, Woodcock JA, Clohisy JC, et al. The Shenton line in the diagnosis of acetabular dysplasia in the skeletally mature patient. J Bone Joint Surg Am 2011;93(Suppl 2):35–39. DOI: 10.2106/JBJS.J.01717.

ORIGINAL RESEARCH

A High-throughput Analysis of Gene Expression in the Intestine in Severe Neonatal Anemia

Jayanta K Das[1,2$], Akhil Maheshwari[2–19]

Received on: 16 October 2025; *Accepted on*: 18 December 2025; *Published on*: 15 January 2026

Abstract

Background: In recent years, most centers caring for premature and critically ill infants have adopted restrictive red blood cell (RBC) transfusion guidelines to minimize transfusions in asymptomatic infants unless the hematocrit drops below 20–22%. However, there is renewed awareness about the risks of anemia in neonates. We have shown that severely anemic human infants show higher intestinal permeability, bacterial translocation, and both local/systemic inflammation. In this study, we used an established mouse pup model to investigate the effect of severe anemia in the intestine using an open-ended, high-throughput approach using microarrays.

Materials and methods: C57BL/6 mouse pups were rendered severely anemic (hematocrits = 20–24%, *n* = 14 in control and anemic arms) by serial phlebotomies on postnatal days 2–10. The ileocecal region was processed to extract mRNA, and gene expression was measured using open-ended, high-throughput microarray analyses, using a threshold for altered gene expression of ±1.5-fold. To ensure the effectiveness of the analyses and avoid unknown errors, the authors compiled their own robust bioinformatics pipelines for microarray data processing, principal component analysis (PCA), screening of differentially expressed genes (DEGs), hierarchical clustering analysis, gene-set enrichment analysis, and quantitative analysis.

Results: We first conducted PCA using log-transformed gene expression data. The visualization of a few principal components (PCs) in intestinal tissue samples after batch-effect correction showed a distinct separation between control and anemia groups. Comparison of the anemic vs control samples showed 2,826 DEGs, with 1,041 upregulated and 1,825 downregulated genes. Enrichment analyses were conducted, targeting gene ontology (GO) for biological processes (BPs), molecular functions (MFs), and cellular components (CC). Two major pathway analyses were also used. The study showed that severe neonatal anemia has a significant biological impact in the gastrointestinal tract, particularly on the maintenance of epithelial cell integrity and gut leakiness, local innate immune responses, and inflammation.

Conclusions/significance: We reviewed the upregulated and downregulated DEGs in this neonatal mouse model of severe anemia. However, even though many of these genes exert similar-looking pathophysiological effects, the reasons for some being induced and others being suppressed were not readily evident. Similarly, the patterns seen in altered expression of some genetic pathways identified in ontology analyses could not be explained. The stage of development, and in humans, transfused blood (composition of the hemoglobin with adult and fetal isoforms) can be important confounding factors and need study. Overall, severe anemia induces significant pathophysiological changes in gut barrier function, innate immune responses, and inflammation.

Keywords: Adenylate kinase 4, Atos homolog A, Bioinformatics, Carbamoyl phosphate synthetase 1, Caspase-1, Critical developmental epochs, CSA-conditional, Cyclin G2, Cytochrome P450 family 27 subfamily A polypeptide 1, Dyskeratosis congenita 1, Erythropoietin, Fatty acid binding protein 4, Fetal hemoglobin, FITC-dextran, FK506 binding protein 5, Gene expression noise, Gene ontology, Gene Set Enrichment Analysis, Hierarchical clustering of differentially expressed genes, Highly transfused population, High-throughput approach, Homeobox C6, Hyaluronan and proteoglycan link protein 1, Infant, Inflammation, Integrin α2, Intestinal development, Intestine, Ki-67, Kinesin 11, Kinesin 4, k-mer, Kyoto Encyclopedia of Genes and Genomes, Linear models for microarray data, Log2 transformation, Log-fold change, Matrix Gla protein, MEF2B, Metadata, Mitogen-activated protein kinase 1 interacting protein 1, Multi-array Average, Myelin basic protein, Myocyte enhancer factor 2B (MEF2B), NADPH oxidase 4, NADPH oxidase activator 1, Necrotizing enterocolitis, Neonatal anemia, Neuregulin 1, Newborn, Nucleotide sequence, Oxygen affinity, Phlebotomy, Physiological nadir, Pipelines, Principal component analysis, Proliferation-associated 2G4, Prolyl endopeptidase, Protein arginine N-methyltransferase 5, Reactome, Red blood cell life span, Red blood Let it be, Robust multi-array average, RStudio, Software applications, Soluble (GPT/ALT, cytosolic alanine aminotransferase), Solute carrier family 17 member 8, Solute carrier family 23 member 4, Splanchnic perfusion, T cell activation-dependent protein, Thyrotroph embryonic factor, Vascular endothelial growth factor A, Volcano plot, Zinc finger and BTB domain containing 16.

Newborn (2025): 10.5005/jp-journals-11002-0145

Key Points

- Most premature/critically ill infants develop moderate–severe anemia in the first few weeks after birth. Several factors suppress erythropoiesis, and there are also phlebotomy losses.
- There is renewed awareness about the risks of anemia in neonates. However, considering the risks, current guidelines discourage transfusions in asymptomatic infants until the hematocrit drops to 20–22%.
- C57BL/6 mouse pups were rendered severely anemic (hematocrits = 20–24%, *n* = 14 in control and anemic arms) by

[1]Department of Pediatrics, Johns Hopkins University School of Medicine, Baltimore, Maryland, United States of America

[2]Global Newborn Society, Harrison, New York, United States of America

[3]GNS Forum for Transgenerational Inheritance, Harrison, New York, United States of America

[4]Department of Pediatrics/Neonatology, Boston Children's Health Physicians Group at the Maria Fareri Children's Hospital, New York Medical College, Valhalla, New York, United States of America

[5]Mongolian Association of Obstetrics, Gynecology, and Neonatology, UlaanBaatar, Mongolia

serial phlebotomies. The ileocecal region was harvested on postnatal day 11, and gene expression was measured using an open-ended, high-throughput microarray analysis. To ensure the effectiveness of the analyses, the authors compiled their own robust bioinformatics pipelines.

- We detected 2,826 differentially expressed genes (DEGs), with 1,041 upregulated and 1,825 downregulated genes. Severe neonatal anemia had a significant biological impact on the gastrointestinal tract, particularly in maintaining of epithelial cell integrity and gut leakiness, local innate immune responses, and inflammation.
- Severely anemic animals, even when apparently asymptomatic, showed altered expression of a large number of genes. However, even though many of these genes were visualized as likely aligned in pathophysiological effects, the reasons for some being induced and others being suppressed were not readily evident. Further studies are needed.

Introduction

In most premature and critically ill term infants, the normal postnatal nadir in blood hemoglobin levels may begin sooner after birth, and may also be more pronounced and prolonged.[1–4] Diverse factors, such as suppression of bone marrow activity following exposure to high ambient oxygen concentrations in the *ex utero* environment, blunted erythropoietin response due to immaturity of the kidneys and liver, and short life-span of immature red blood cells (RBCs) have been implicated.[5] Compared to adults, neonatal RBCs show a shorter life span; those from term neonates last only for 60–90 days, and the ones from premature infants for 35–50 days.[6] Low membrane elasticity/deformability, altered cell sphericity (surface area-to-volume ratio), and high internal (cytoplasmic) viscosity are some factors that increase hemolysis during passage through small-lumen vessels.[7] In addition to these limitations intrinsic to RBCs, rapid somatic growth with expansion of the blood volume is another reason.[8] Nutritional factors can also contribute to anemia; preterm infants have low iron stores, as most maternal–fetal iron transfer takes place during the third trimester.[9,10]

Severe anemia can have serious consequences because it reduces the blood's ability to carry oxygen to vital organs.[5] In adult subjects, this can lead to easy fatiguability, shortness of breath, and dizziness, and in severe cases, myocardial dysfunction. In infants and children, severe anemia can impair hemodynamic stability, increase the risk of infections, and delay development.[5,11] With this scientific background, premature and critically ill infants became one of the most highly transfused groups of patients in any hospital.[12] However, as more information became available about the risks of RBC transfusions, the pendulum of medical opinions swung in the other direction. Current guidelines recommend withholding transfusions in growing premature/critically ill infants unless they develop clinical signs or the hematocrit drops as low as 20–22%.[13]

In recent years, concerns are again emerging about whether our transfusion thresholds could have become far too restrictive without adequate data on safety. There is a possibility of harm from severe anemia during critical developmental epochs. There have been clinical quality-improvement efforts and technological advances to reduce phlebotomy losses.[4,14] Studies have shown that severe anemia may divert, even though suboptimally, blood flow to critical organs such as the brain, heart, and the adrenal glands from others such as the gastrointestinal tract.[15,16] In addition to ischemic effects, anemia may also increase the risk of inflammation both at a systemic level and in organs such as the gastrointestinal tract.[17,18] To investigate the impact and mechanisms of severe anemia-induced changes in the intestine, we have developed a murine pup model where we perform repeated phlebotomies to induce consistent, measurable anemia.[19,20] There is a possibility that the developing intestine might be more susceptible to severe anemia because (1) splanchnic perfusion may get diverted to other vital organs; (2) higher risk of ischemic injury due to limited inter-regional and collateral perfusion; and (3) higher oxygen affinity of fetal hemoglobin that can restrict tissue oxygen delivery.[21–24] In mice, the intestine shows ontogenic delay at birth; it begins to mature similar to full-term human infants only by postnatal day (P) 16–18.[19,20,25–27] To induce anemia similar to that seen in premature/critically ill human infants, we perform five measured phlebotomies on postnatal days 2–10 and the hematocrit drops from the 35–40% at birth to the 20–24% seen in severely anemic human infants.[20] Similar to humans, these anemic pups show

[6]Department of Neonatology, Institute of Maternal and Child Health, Matuail, Dhaka, Bangladesh

[7]Dr. Mozib Newborn Foundation, Dhaka, Bangladesh

[8]Pioneers—Looking for Sustainable Ways to Reduce Infant Mortality, Oslo, Norway

[9]Banaras Hindu University Institute of Excellence, Varanasi, Uttar Pradesh, India

[10]S.A.B.R.E.E. Enrichment Academy, Saint Louis, Missouri, United States of America

[11]The Skylar Project, Daphne, Alabama, United States of America

[12]International Society for Marginalized Lives, Harrison, New York, United States of America

[13]PreemieWorld Foundation, Springfield, Virginia, United States of America

[14]Carlo GNS Center for Saving Lives at Birth, Birmingham, Alabama, United States of America

[15]Autism Care Network Foundation, Chandigarh, India

[16]Neonatology-Certified Foundation, Brooksville, Texas, United States of America

[17]GNS Infant Nutrition Education Program, Harrison, New York, United States of America

[18]International Prader-Willi Syndrome Organization, Cambridge, United Kingdom

[19]First Breath of Life, Shreveport, Louisiana, United States of America

[$]**Current affiliation:** JDK is currently affiliated with the Longitudinal Studies Section, Translation Gerontology Branch, National Institute on Aging, National Institutes of Health, Baltimore, Maryland, United States of America

Corresponding Author: Akhil Maheshwari, Department of Pediatrics/Neonatology, Boston Children's Health Physicians Group at the Maria Fareri Children's Hospital, New York Medical College, Valhalla, New York, United States of America, Phone: +1-708-910-8729, e-mail: akhil@globalnewbornsociety.org

How to cite this article: Das JK, Maheshwari A. A High-throughput Analysis of Gene Expression in the Intestine in Severe Neonatal Anemia. Newborn 2025;4(4):165–177.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as the Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of Editor-in-Chief and his research group.

increased intestinal permeability, inflammation, and if transfused, necrotizing enterocolitis-like gut injury.[19,20]

In this study, we compared gene expression in the ileocecal tissue in control and severely anemic pups using open-ended, high-throughput microarray analyses. The aim was to characterize the changes in gene expression in normal vs age-matched anemic mouse pups.[19,20] Because most (>95%) severely anemic human infants/murine pups do not show clinically manifest intestinal complications, we chose an altered gene expression threshold of ±1.5-fold, lower than the usual ±2-fold.[19,20,28] Some "noise" was anticipated in gene expression, and therefore, we studied a larger cohort compiled of pups from two successive deliveries.

Despite the progress in developing/implementing the tools for bioinformatics analyses over the last decade, there are continuing challenges.[29] Most software applications/pipelines have been compiled from diverse sources; there have been incongruent statistical and algorithmic approaches, multiple professional philosophies, diverse academic institutions, and inconsistent modifications over time.[30,31] Hence, despite all achievements, concerns remain. Bioinformatics pipelines developed even with mainstream scientific tools can be suboptimal in analysis provenance, such as in application versioning for tracking of metadata or in handling of intermediate data, including temporary and log files.[32] There is a massive expansion of data during processing, which is often stored in obscure folders within the bioinformatics applications.[33] High-throughput sequencing experiments also generate massive raw data in text-based files containing nucleotide sequence and quality score information.[34] To ensure that we generate useful knowledge from these data, we trimmed and cleaned raw data files before secondary analysis and alignment to a reference genome, *de novo* assembly, or k-mer counting.[35–37] These steps also generate massive secondary and intermediate files describing the alignment, assembly, and/or quantification of data. Hence, these derived files were sorted, filtered, and annotated prior to analysis. Considering the complexity of these constructs, we reviewed and evaluated all command-line instructions to ensure reproducibility of data in each step in this analysis.[38] The details of these efforts can be seen in the methods section of this manuscript.

Materials and Methods

Animals

Animal studies were approved by the Institutional Animal Care and Use Committee and complied with the NIH guide for the care and use of laboratory animals. We used two successive cohorts of C57BL/6 mouse litters, with 12 and 16 pups, respectively.[39] The laboratory personnel who reared the animals were blinded, and the pups were randomized independently to form two groups, each with 14 animals. The pooling of two batches from successive deliveries can be justified as the C57BL/6 mice in our laboratory are obtained from a single source and the animals are highly inbred. Thus, anemia in the study groups can be safely ascribed to a single intervention, phlebotomy, with no unknown confounding factors such as hemolysis.[20] The pooling of highly similar mouse cohorts is scientifically appropriate, and the "noise" in comparison of gene expression was likely minimal.

In the anemic group, mice were subjected to facial vein phlebotomy to collect 40 μL blood on postnatal day (P) 2, P4, P6, P8, and P10; 5 μL blood was diluted 1:20 in Cellpak reagent (Sysmex, Kobe, Japan) and analyzed using the Sysmex XT-2000iV veterinary hematology analyzer. The hematocrits consistently dropped to the 20–24% range after 5 phlebotomies; hematocrits, RBC indices, and reticulocyte hemoglobin were monitored at each blood draw to ensure standardization. To further confirm the systemic effects of severe anemia, intestinal permeability was measured on P11 by administering FITC-dextran (10 kDa, 400 mg/kg) by gavage, followed by measurement of the fluorescence signal in plasma 4h later.[40] Animals were then sacrificed, and the ileocecal regions were harvested for mRNA extraction.

Microarray Data Processing

We measured gene expression in the harvested ileocecal tissues by RNA microarray and used quantitative polymerase chain (qPCR) reactions to align the reads. The raw data (.CEL files) were pre-processed using the bioconductor package "oligo," an established algorithm [the function *read.cellfiles()*] in the software environment *R* that helps read the intensity files in the native format. The gene expression profiles of anemic and control pups were then compared using the Robust multi-array average (RMA) normalization, which helps in (1) background noise removal; (2) quantile normalization; and (3) log2 transformation of expression values.[41–47] To identify differentially expressed genes (DEGs), we followed a standard approach in the bioconductor package, the linear models for microarray data (LIMMA).[48] The *lmfit()* function here can corroborate with other packages, and the *ebayes()* function (empirical Bayes statistics) can "squeeze" genewise residual variances to help identify DEGs.[49]

We compared the gene expression profiles between the control and anemic animals. The LIMMA [*removeBatchEffect()*] function in *R* can help mitigate possible batch effects related to hybridization time or other technical variables.[50] It enhances the differential power analysis in RNA sequencing and microarray studies. Overall, the goal of using multiple computational approaches was to minimize the "noise" related to technical factors when combining multiple batches to identify DEGs.[51] We utilized the program Genome, version: mm10 (*Mus musculus*) and the Affymetrix Mouse Transcriptome Array 1.0 (Annotation: MTA-1_0.r3.na36.mm10.a1.transcript.csv) for mapping probe sets to gene symbols and other annotations. This comprehensive approach helped align our experimental data with the latest genomic information.

Principal Component Analysis, Screening of DEGs, and Hierarchical Clustering Analysis

We identified DEGs based on statistically significant differences in expression, which were identified using both a *p*-value and a log-fold change |log(FC)|. The LIMMA approach calculates the log-fold change on log-transformed data, expressed as "Log(FC)" = mean[log2(Group I)] – mean[log2(Group II)], which is distinct from the actual log2(FC) = log2[mean(Group I/Group II)].[48] A log-fold change for any gene greater or less than [|log(FC)| ≥ 0] was considered significant. Statistical significance was identified at $p < 0.01$. This analytical flow helped in the identification of DEGs with high cut-off criteria for both fold changes and statistical confidence.

Significantly altered DEGs were further subjected to sample-level quality control (QC) through principal component analysis (PCA).[52] This allowed us to qualitatively assess the closeness of clustering of our replicates. We also used hierarchical clustering methods to confirm that the identified variations in our data actually arose in experimental conditions or were due to other

factors.[53] Here, visual impressions of the clusters of over- and under-expressed genes within the groups provided some insights into patterns of gene expression.

Gene-set Enrichment Analysis

We conducted a pre-ranked Gene-set Enrichment Analysis (GSEAPreranked) to identify significantly enriched genes in various functional and biological pathways.[54,55] The GSEA allows systematic exploration of enriched genes based on their positions in the ranked list, providing valuable insights into the functional significance of gene expression patterns.[56] In our analysis, genes were ranked based on a combination of *p*-value and the sign of log-fold changes. This ranking positioned over-expressed genes at the top and under-expressed genes at the bottom. In these analyses, we focused on the Molecular Signature Database (https://www.gsea-msigdb.org/gsea/index.jsp) with the three gene ontology (GO) sets, the GO biological process (BP) with 7,647 gene sets, the GO cellular component (CC) with 1,015 gene sets, and the GO molecular function (MF) with 1,799 gene sets. Also, we used two databases, the KEGG (Kyoto Encyclopedia of Genes and Genomes) with 619 gene sets and the *Reactome* with 1,692 gene sets for pathway analysis. Finally, we sought to examine whether GSEAPreranked could help determine whether an *a priori*-defined set of genes exhibited statistically significant up- or downregulation of expression. To establish statistical significance, we used a false discovery rate (FDR)-corrected threshold of $p < 0.05$.[57–64]

Quantitative Analysis, Visualization, and Statistical Analysis Software Tools

Quantitative analyses were conducted in RStudio, utilizing *R* version 4.3.1 (https://www.r-project.org/).[65] Visualization and plot generation were accomplished using "ggplot" and associated functions in *R*.[66,67] Relevant statistical measures were carried out as outlined in the text as needed. Raw data have been deposited at Gene Expression Omnibus (GEO) under accession number GSE94292.

Results

Microarray Profiles between Anemia and Control Groups

We first conducted PCA using log-transformed gene expression data from the entire array list. The visualization of a few principal components (PCs) in intestinal tissue samples after batch-effect correction showed a distinct separation between control and anemia groups ($n = 14$ in each group; Fig. 1). Most control pups showed a closer proximity in expression than the anemic mice, although a few outliers were seen closer in the two groups. This observation aligns with the view that severe neonatal anemia might represent an exaggerated physiological nadir in hemoglobin/hematocrit levels rather than a true disease.

Identification of DEGs and Interpreting Results

We next compared gene signal intensities between the anemia and control groups. To identify DEGs, we used our established criteria of $p < 0.01$ and $|FC| > 0$. Comparison of the anemic vs control samples showed 2,826 DEGs, with 1,041 upregulated and 1,825 downregulated genes (volcano plot in Fig. 2). The larger number of downregulated DEGs could possibly explain the clinical impression that many physiological activities are suppressed in severe anemia.

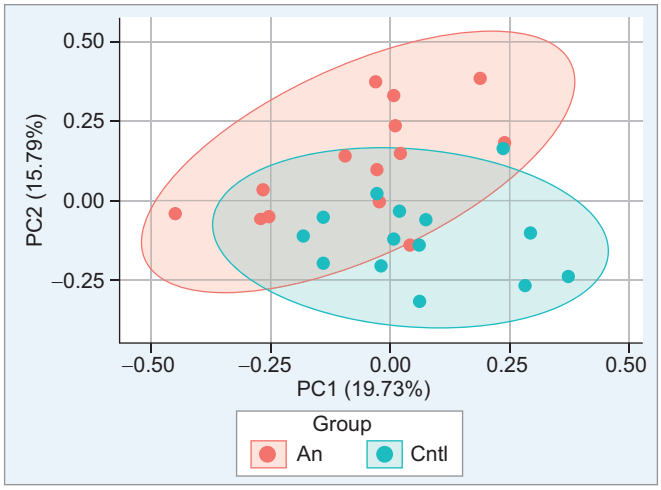


Fig. 1: Principal component analysis shows the distribution of anemia (An) and control (Cntl) mice. The data have been depicted after batch effect correction

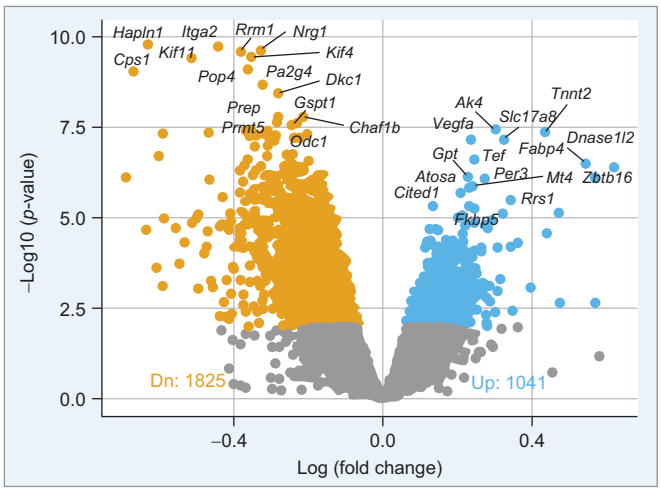


Fig. 2: Volcano plot shows downregulated (orange) and upregulated (blue) genes in the intestine in anemic mice. To identify DEGs, we used established criteria of $p < 0.01$ and $|FC| > 0$

We next performed hierarchical clustering of DEGs in anemic vs control pups. Figure 3 shows hierarchical clustering of the top 50 genes. Comparisons of DEGs selected for statistically significant differences in expression helped refine the results. The top 40 genes, ranked by *p*-value, are listed in Table 1.

Functional Enrichment Analysis of DEGs

Enrichment analyses were conducted, targeting gene ontology (GO) for GO-BP, GO-MFs, and GO-CCs (GO-CC; Fig. 4). Two major pathways (reactome and KEGG) were also studied (Fig. 5). Gene ontology terms and pathways were chosen based on an adjusted *p*-value threshold of *q*-value < 0.05, and were prioritized according to the net enrichment score (NES; top-down). Major alterations were seen in BP, CC, and MFs.

Discussion

We present a detailed investigation of the effects of severe anemia on gene expression in an established mouse pup model.[19,20,68]

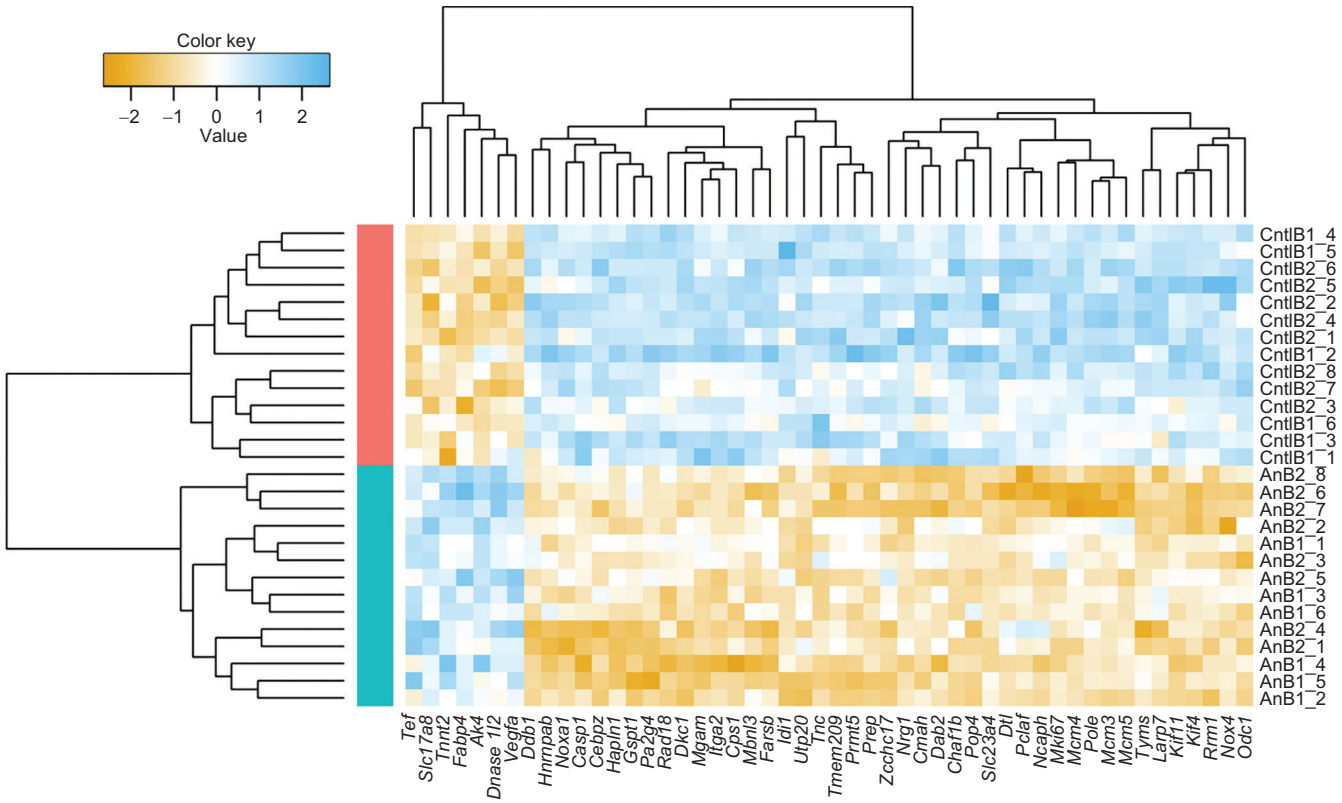


Fig. 3: Hierarchical clustering of DEGs with statistically significant differences ($p < 0.01$ and $|FC| > 0$). The figure shows downregulated (orange) and upregulated (blue) genes in C57B6 mice (B) in anemia (An), control (Cntl) groups. The intensity of the color shows the degree of significance

Table 1: Top 20 upregulated and 20 downregulated genes in severe anemia, ranked by *p*-value. The table also shows the log fold changes

S. No.	*Gene symbol*	*Gene full name*	*logFC*	*p-value*
A. Upregulated genes				
1.	*Dnase1l2*	Deoxyribonuclease 1-like 2	0.619347	3.95E-07
2.	*Zbtb16*	Zinc finger and BTB domain containing 16	0.569237	8.18E-07
3.	*Fabp4*	Fatty acid binding protein 4, adipocyte	0.544753	3.17E-07
4.	*Olr1*	Oxidized low density lipoprotein (lectin-like) receptor 1	0.470712	7.01E-06
5.	*Tnnt2*	Troponin T2, cardiac	0.435355	4.37E-08
6.	*Rrs1*	Ribosome biogenesis regulator 1	0.341766	3.36E-06
7.	*Slc17a8*	Solute carrier family 17 member 8	0.326098	6.66E-08
8.	*F13a1*	Coagulation factor XIII, A1 subunit	0.319597	7.66E-06
9.	*Ak4*	Adenylate kinase 4	0.300943	3.74E-08
10.	*Per3*	Period circadian clock 3	0.27136	8.39E-07
11.	*Cyp27a1*	Cytochrome P450, family 27, subfamily a, polypeptide 1	0.245001	5.48E-06
12.	*Tef*	Thyrotroph embryonic factor	0.24359	2.46E-07
13.	*Mt4*	Metallothionein 4	0.240332	1.3E-06
14.	*Vegfa*	Vascular endothelial growth factor A	0.23759	6.93E-08
15.	*Atosa*	Atos homolog A	0.233059	1.48E-06
16.	*Fkbp5*	FK506 binding protein 5	0.231856	4.65E-06
17.	*Gpt*	Glutamic pyruvic transaminase, soluble	0.227797	7.21E-07
18.	*Vwf*	von Willebrand factor	0.212191	8.51E-06
19.	*Cited1*	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	0.210201	2.01E-06
20.	*Mbp*	Myelin basic protein	0.135204	4.93E-06
B. Downregulated genes				
1.	*Cps1*	Carbamoyl-phosphate synthetase 1	−0.66876	9.23E-10
2.	*Hapln1*	Hyaluronan and proteoglycan link protein 1	−0.63026	1.63E-10

(Contd...)

Table 1: *(Contd...)*

S. No.	Gene symbol	Gene full name	logFC	p-value
3.	*Casp1*	Caspase 1	–0.58935	4.74E-08
4.	*Kif11*	Kinesin family member 11	–0.51347	3.9E-10
5.	*Mki67*	Antigen identified by monoclonal antibody Ki 67	–0.46841	4.46E-08
6.	*Itga2*	Integrin alpha 2	–0.44338	1.87E-10
7.	*Rrm1*	Ribonucleotide reductase M1	–0.38166	2.64E-10
8.	*Slc23a4*	Solute carrier family 23 member 4	–0.36232	4.06E-08
9.	*Pop4*	Processing of precursor 4, ribonuclease P/MRP family, (*S. cerevisiae*)	–0.36144	8.16E-10
10.	*Noxa1*	NADPH oxidase activator 1	–0.35592	3.48E-08
11.	*Kif4*	Kinesin family member 4	–0.35256	3.62E-10
12.	*Nrg1*	Neuregulin 1	–0.32617	2.37E-10
13.	*Pa2g4*	Proliferation-associated 2G4	–0.3241	2.13E-09
14.	*Nox4*	NADPH oxidase 4	–0.3092	3.83E-08
15.	*Prmt5*	Protein arginine N-methyltransferase 5	–0.28264	2.61E-08
16.	*Dkc1*	Dyskeratosis congenita 1, dyskerin	–0.28244	3.74E-09
17.	*Prep*	prolyl endopeptidase	–0.27999	1.53E-08
18.	*Gspt1*	G1 to S phase transition 1	–0.24252	2.75E-08
19.	*Odc1*	Ornithine decarboxylase, structural 1	–0.23043	2.37E-08
20.	*Chaf1b*	Chromatin assembly factor 1, subunit B (p60)	–0.21507	1.58E-08

Severe neonatal anemia has a significant impact on epithelial cell integrity and gut leakiness, local innate immune responses, and inflammation. These results are consistent with our previously reported results that this severity of anemia (lowest hematocrits of 20–24%) was associated with increased permeability to food-borne macromolecules and enteral bacteria, and with mucosal inflammation with macrophage infiltration.[19] We have previously documented ultrastructural and molecular changes underlying these effects.[19,20] The pathophysiological effects were specific for postnatal age and the intestinal permeability continued to be high until P14; there was then a gradual recovery of the barrier function by P20. Adult mice with comparable low hematocrits showed only minimal physiological changes.[20]

A number of DEGs were identified; there were 1,041 upregulated and 1,825 downregulated genes (Fig. 2). The larger number of downregulated DEGs is consistent with the possibility of many physiological activities being suppressed in severe anemia. Hierarchical clustering shows genes with similar patterns of expression (co-expression; Fig. 3).[69] We tried to identify genes/clusters with shared biological functions based on distance metrics but could not see clear patterns.[70] The list of DEGs shown in Table 1 shows many regulatory genes that are likely involved in more than one cellular function and are not specific to anemia.[71]

We reviewed the upregulated and downregulated DEGs in some detail. However, even though many of these genes exert similar-looking pathophysiological effects, the reasons for some being induced and others being suppressed are not readily evident. The zinc finger and BTB domain containing 16 (ZBTB16) influences epithelial and immune regulation by altering transcriptional control of cell differentiation and inflammatory responses.[72,73] It promotes innate cytokine responses in local leukocytes.[73] Increased cyclin G2 may uniquely help maintain epithelial homeostasis in response to nutrient deprivation, DNA damage, or inflammatory stress. Adenylate kinase 4 (AK4) may reflect a stress-adaptive response in the mitochondria, where it might help regulate nucleotide homeostasis and cellular survival pathways.[74–76] Increased mitogen-activated protein kinase 1 interacting protein 1 (MAPK1IP1) is likely another stress response as it enhances MAPK/ERK signaling in IEC proliferation and differentiation.[77] Increased cytochrome P450 family 27 subfamily A polypeptide 1 (CYP27A1) is also likely to be a mitochondrial compensatory response as it alters bile acid and cholesterol metabolism; it might promote mucosal homeostasis but may also have inflammatory responses.[77] Upregulation of myocyte enhancer factor 2B (MEF2B) is also likely to promote transcriptional networks needed to regulate epithelial cell survival, differentiation, and immune signaling.[78,79]

Thyrotroph embryonic factor (TEF) is a transcription factor involved in stress adaptation. It can enhance nutrient absorption, epithelial turnover, and barrier maintenance. Similarly, increased metallothionein 4 (MT4) also represents a stress-adaptive response. Increased vascular endothelial growth factor A (VEGFA) drives angiogenesis and vascular remodeling in the intestine.[80–82] In acute injury, VEGFA supports mucosal healing by enhancing blood supply, nutrient delivery, and epithelial regeneration. However, excessive VEGFA signaling can promote leaky, immature blood vessels, and increase mucosal edema and amplify inflammation. Atos homolog A (ATOSA), glutamic pyruvic transaminase, soluble (GPT/ALT, cytosolic alanine aminotransferase), and CSA-conditional, T cell activation-dependent protein (CSADP) are conserved stress-adaptive compensatory mechanisms involved in epithelial cell survival/proliferation.[83–86] Increased homeobox C6 (HOXC6) and MEF2B can activate epithelial proliferation, differentiation, and remodeling. Similarly, induction of matrix Gla protein, troponin T2, cardiac (TNNT2), and ribosome biogenesis regulator 1 (RRS1) represent cellular stress responses. Fatty acid binding protein 4 (FABP4, adipocyte FABP) can activate NF-κB and PPARγ-mediated inflammatory signaling in gut macrophages.[87–91]

Solute carrier family 17 member 8 (SLC17A8) activates a vesicular glutamate transporter in select neurons and non-neuronal cells.[92] Its upregulation may cause gut dysmotility, barrier dysfunction, and mucosal inflammation. Similarly, myelin basic protein (MBP) is upregulated on local neurons and infiltrating immune cells during

Fig. 4: Changes in GO pathways in severe anemia. GO-BP, gene ontology-biological process; GO-CC, gene ontology-cellular component; GO-MF, gene ontology-molecular function

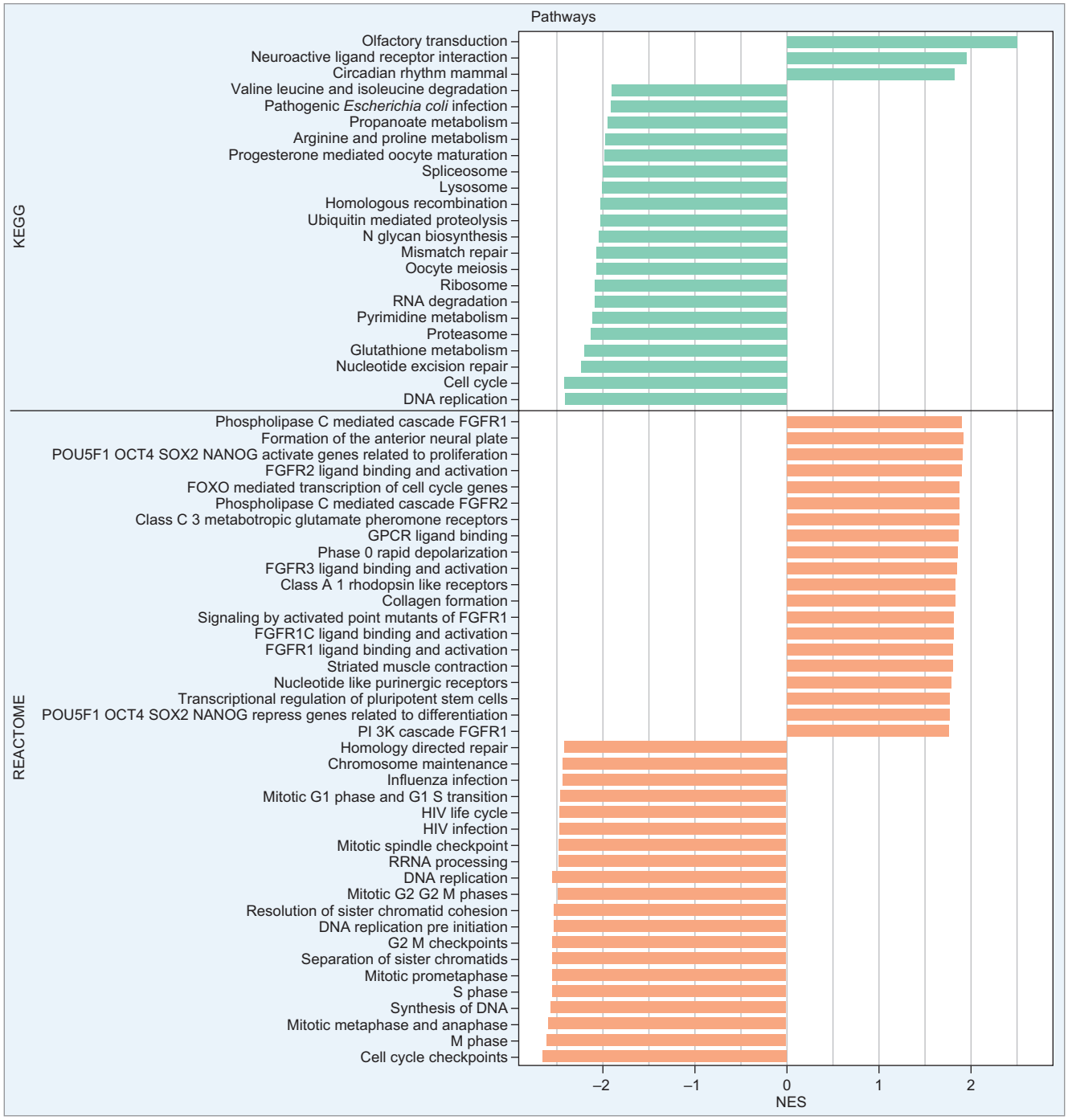


Fig. 5: Changes in GO pathways in severe anemia. We used two databases, the KEGG with 619 gene sets and the *Reactome* with 1,692 gene sets for pathway analysis

inflammation.[93] Increased FK506 binding protein 5 (FKBP5) reflects heightened sensitivity to stress and dysregulated glucocorticoid signaling.[94] Its upregulation in intestinal epithelial and immune cells has been linked with altered NF-κB activation, oxidative responses, and apoptosis regulation.

Several genes were downregulated, possibly increasing the risk of mucosal injury, bacterial translocation, and consequently, local/systemic inflammation. Decreased carbamoyl phosphate synthetase 1 (CPS1) in mitochondria can impair the detoxification of ammonia and increase enterocyte apoptosis and, consequently, gut barrier dysfunction.[95] Downregulation of hyaluronan and proteoglycan link protein 1 (HAPLN1) can disrupt the extracellular matrix and gut barrier.[96,97] Caspase-1 normally cleaves pro-IL-1β and pro-IL-18 into their active cytokine forms, which promote protective immune responses, epithelial integrity, and antimicrobial defense. Kinesins 4 and 11 (KIF4, KIF11) are needed for epithelial cell proliferation and maintenance of barrier integrity.[98–101] Ki-67 expression is a marker of epithelial cell regeneration. Integrin α2

(ITGA2) is a collagen receptor that helps anchor gut epithelial cells, and the proliferation-associated 2G4 (PA2G4, also known as ErbB3-binding protein 1, EBP1) helps maintain the epithelial barrier. Ribonucleotide reductase M1 (RRM1) is needed for epithelial cell proliferation in the crypts. Precursor 4, ribonuclease P/MRP, normally promotes the maturation of noncoding RNAs required for protein synthesis and mitochondrial function.[102–106]

NADPH oxidase activator 1 (NOXA1) and NADPH oxidase 4 (NOX4) normally help maintain the NADPH oxidase complexes and reactive oxygen species (ROS) generation. Neuregulin 1 (NRG1) is needed for trophic and protective signaling needed for epithelial–neuronal interactions.[107–109] Decreased protein arginine N-methyltransferase 5 (PRMT5) is an important epigenetic and post-transcriptional regulator that influences gene expression, RNA splicing, and cell cycle control.[110] In the gut epithelium, decreased PRMT5 may impair epithelial renewal, weaken regenerative capacity after injury, and alter immune signaling pathways. Solute carrier family 23 member 4 (SLC23A4) is required for maintaining redox balance in the epithelium.[111] Dyskeratosis congenita 1 (DKC1, dyskerin) protein is a nucleolar protein essential for telomerase RNA stabilization, ribosomal RNA modification, and telomere maintenance.[112] It is needed for epithelial cell survival and renewal. Decreased prolyl endopeptidase (PREP, also called prolyl oligopeptidase) in the intestine may interfere with normal peptide processing and signaling within the gut. Prolyl endopeptidase is a serine protease that cleaves small proline-containing peptides, influencing the activity of neuropeptides, peptide hormones, and signaling molecules important for gut motility, mucosal immunity, and epithelial function.[113]

These patterns seen in altered in the expression of these genes showed some, but not a perfect, match with gene sets found enriched in subsequent ontology analyses. In the gene ontology analyses, anemia was more likely to affect some specific pathways; GO-BP, GO-CC, and GO-MF showed neuronal function/differentiation, cell division, motility, and fat/protein synthesis as more likely to be affected (Fig. 4).[114] The pathway analyses seemed to broadly follow similar broad patterns (Fig. 5). Considering the lack of a readily evident synchrony between the DEGs identified in our earlier analyses and the later quest for patterns, we needed to consider a few possibilities: (1) the identified regulatory genes need further characterization and could very well be also involved in more than one signaling pathways/cellular functions; (2) the lists of genes and pathways might not be congruous because the two have been compiled based on the magnitude of statistical significance, not cellular functions/potency; and (3) not all isoforms of the proteins have yet been identified and there might be other gene sequences that need to be characterized.[115–117]

The strengths of this study include a well-standardized animal model, where the severity of anemia has numerical and functional similarities to that seen in premature and critically ill human infants. Hemoglobin is a fairly well-conserved protein during evolution, and so such pre-clinical studies can provide a strong scientific basis for further clinical studies. Furthermore, at least in the intestine, mouse pups resemble mid-gestation human infants and so the model is appropriate for studying the effects of severe anemia in premature infants.[118–119]

The weakness of the study is that, unlike humans, mice have only one type of hemoglobin.[120] Growing human infants, particularly those who have been transfused with adult blood, carry a mixture of fetal (HbF) and adult (HbA) hemoglobins that have different oxygen-carrying capacities.[121] Furthermore, to ensure that the model is reproducible, anemia is induced using only a single method, serial phlebotomies.[19,20] The impact of phlebotomy beginning in a 2-day-old mouse pup could be similar to anemia beginning in an extremely premature infant that continues until birth but with opportunities for hemodynamic compensation. Hence, there is a need for different models to represent other causes of neonatal anemia, such as hemolysis. Another point is the source of experimental animals.[122] In our laboratory, we have cautiously sourced all our animals from a single commercial repository and tried to maintain consistency in maternal feedings, temperature, and nesting material/hay. In some of these issues, multigenerational studies can help in obtaining larger sample sizes and minimize some transient epigenetic/environmental confounders.[123] Hence, we included two successive cohorts of pups in this study. We have still not tested the microflora and plan to do so in future studies.[124,125]

To conclude, this study adds more evidence to our earlier studies that severe anemia induces pathophysiological changes in the neonatal intestine with altered barrier function, local innate immune responses, and inflammation.[19,20] The hemoglobin thresholds at which anemia alters function likely vary between organs; our previously published data show effects in the brain at higher hemoglobin concentrations.[15,16] Previous history of transfusions is another potential source of bias; fetal and adult hemoglobin show considerable differences in function and so the threshold for intervention will likely differ in previously transfused infants.[126–128] The task is complicated in infants and needs caution before making universal recommendations.[129,130]

Acknowledgments

The authors would like to express their gratitude to Dr Mohan K Krishnan and Late Dr Kopperuncholan Namachivayam for their efforts in the development of the animal model. The software programs used in the current manuscript were developed after JKD had left the Johns Hopkins University School of Medicine.

Grant Support

The study was done in years 1–3 of National Institutes of Health award HL124078 (to A.M.)

Author Contributions

AM and JKD designed and performed the study, and wrote the manuscript.

Orcid

Akhil Maheshwari https://orcid.org/0000-0003-3613-4054

References

1. DeMaeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. World Health Stat Q 1985;38(3):302–316. PMID: 3878044.
2. Brabin BJ, Premji Z, Verhoeff F. An analysis of anemia and child mortality. J Nutr 2001;131(2S-2):636S–645S. DOI: 10.1093/jn/131.2.636S.
3. Strauss RG. Anaemia of prematurity: Pathophysiology and treatment. Blood Rev 2010;24(6):221–225. DOI: 10.1016/j.blre.2010.08.001.
4. Bateman ST, Lacroix J, Boven K, et al. Anemia, blood loss, and blood transfusions in North American children in the intensive care unit. Am J Respir Crit Care Med 2008;178(1):26–33. DOI: 10.1164/rccm.200711-1637OC.

5. Maheshwari A, Patel RM, Christensen RD. Anemia, red blood cell transfusions, and necrotizing enterocolitis. Semin Pediatr Surg 2018;27(1):47–51. DOI: 10.1053/j.sempedsurg.2017.11.009.
6. Pearson HA. Life-span of the fetal red blood cell. J Pediatr 1967;70(2):166–171. DOI: 10.1016/s0022-3476(67)80410-4.
7. Pellegrino C, Stone EF, Valentini CG, et al. Fetal red blood cells: A comprehensive review of biological properties and implications for neonatal transfusion. Cells 2024;13(22):1843. DOI: 10.3390/cells13221843.
8. Kuruvilla DJ, Widness JA, Nalbant D, et al. Estimation of adult and neonatal RBC lifespans in anemic neonates using RBCs labeled at several discrete biotin densities. Pediatr Res 2017;81(6):905–910. DOI: 10.1038/pr.2017.14.
9. German KR, Juul SE. Iron and neurodevelopment in preterm infants: A narrative review. Nutrients 2021;13(11):3737. DOI: 10.3390/nu13113737.
10. German KR, Juul SE. Neonatal anemia. Curr Pediatr Rev 2023;19(4):388–394. DOI: 10.2174/1573396319666221121140627.
11. Martinez-Torres V, Torres N, Davis JA, et al. Anemia and associated risk factors in pediatric patients. Pediatric Health Med Ther 2023;14:267–280. DOI: 10.2147/PHMT.S389105.
12. Aucott SW, Maheshwari A. To transfuse or not transfuse a premature infant: The new complex question. J Perinatol 2019;39(3):351–353. DOI: 10.1038/s41372-018-0306-5.
13. Widness JA, Seward VJ, Kromer IJ, et al. Changing patterns of red blood cell transfusion in very low birth weight infants. J Pediatr 1996;129(5):680–687. DOI: 10.1016/S0022-3476(96)70150-6.
14. Widness JA. Pathophysiology of anemia during the neonatal period, including anemia of prematurity. Neoreviews 2008;9(11):e520. DOI: 10.1542/neo.9-11-e520.
15. Whitehead HV, Vesoulis ZA, Maheshwari A, et al. Progressive anemia of prematurity is associated with a critical increase in cerebral oxygen extraction. Early Hum Dev 2020;140:104891. DOI: 10.1016/j.earlhumdev.2019.104891.
16. Whitehead HV, Vesoulis ZA, Maheshwari A, et al. Anemia and cerebral near-infrared spectroscopy: Should transfusion thresholds in preterm infants be revised? J Perinatol 2018;38(8):1022–1029. DOI: 10.1038/s41372-018-0120-0.
17. Bell EF, Strauss RG, Widness JA, et al. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics 2005;115(6):1685–1691. DOI: 10.1542/peds.2004-1884.
18. Patel RM, Knezevic A, Shenvi N, et al. Association of red blood cell transfusion, anemia, and necrotizing enterocolitis in very low-birth-weight infants. JAMA 2016;315(9):889–897. DOI: 10.1001/jama.2016.1204.
19. MohanKumar K, Namachivayam K, Sivakumar N, et al. Severe neonatal anemia increases intestinal permeability by disrupting epithelial adherens junctions. Am J Physiol Gastrointest Liver Physiol 2020;318(4):G705–G716. DOI: 10.1152/ajpgi.00324.2019.
20. MohanKumar K, Namachivayam K, Song T, et al. A murine neonatal model of necrotizing enterocolitis caused by anemia and red blood cell transfusions. Nat Commun 2019;10:3494. DOI: 10.1038/s41467-019-11199-5.
21. Shah P, Riphagen S, Beyene J, et al. Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. Arch Dis Child Fetal Neonatal Ed 2004;89(2):F152–F155. DOI: 10.1136/adc.2002.023093.
22. Crissinger KD, Granger DN. Characterization of intestinal collateral blood flow in the developing piglet. Pediatr Res 1988;24(4):473–476. DOI: 10.1203/00006450-198810000-00011.
23. Sibbons PD, Spitz L, van Velzen D. Collateral blood flow in the distal ileum of neonatal piglets: A clue to the pathogenesis of necrotizing enterocolitis. Pediatr Pathol 1992;12(1):15–27. DOI: 10.3109/15513819209023278.
24. Brown MS, Phipps RH, Dallman PR. Postnatal changes in fetal hemoglobin, oxygen affinity and 2,3-diphosphoglycerate in previously transfused preterm infants. Biol Neonate 1985;48(2):70–76. DOI: 10.1159/000242156.
25. Nanthakumar NN, Dai D, Meng D, et al. Regulation of intestinal ontogeny: Effect of glucocorticoids and luminal microbes on galactosyltransferase and trehalase induction in mice. Glycobiology 2005;15(3):221–232. DOI: 10.1093/glycob/cwi004.
26. MohanKumar K, Kaza N, Jagadeeswaran R, et al. Gut mucosal injury in neonates is marked by macrophage infiltration in contrast to pleomorphic infiltrates in adult: Evidence from an animal model. Am J Physiol Gastrointest Liver Physiol 2012;303(1):G93–G102. DOI: 10.1152/ajpgi.00016.2012.
27. MohanKumar K, Killingsworth CR, McIlwain RB, et al. Intestinal epithelial apoptosis initiates gut mucosal injury during extracorporeal membrane oxygenation in the newborn piglet. Lab Invest 2014;94(2):150–160. DOI: 10.1038/labinvest.2013.149.
28. Mohamed A, Shah PS. Transfusion associated necrotizing enterocolitis: A meta-analysis of observational data. Pediatrics 2012;129(3):529–540. DOI: 10.1542/peds.2011-2872.
29. Mangul S, Mosqueiro T, Abdill RJ, et al. Challenges and recommendations to improve the installability and archival stability of omics computational tools. PLoS Biol 2019;17(6):e3000333. DOI: 10.1371/journal.pbio.3000333.
30. Halling-Brown M, Shepherd AJ. Constructing computational pipelines. Methods Mol Biol 2008;453:451–470. DOI: 10.1007/978-1-60327-429-6_24.
31. Jay C, Haines R, Katz DS, et al. The challenges of theory-software translation. F1000Res 2020;9:1192. DOI: 10.12688/f1000research.25561.1.
32. Davis-Turak J, Courtney SM, Hazard ES, et al. Genomics pipelines and data integration: Challenges and opportunities in the research setting. Expert Rev Mol Diagn 2017;17(3):225–237. DOI: 10.1080/14737159.2017.1282822.
33. Mallappallil M, Sabu J, Gruessner A, et al. A review of big data and medical research. SAGE Open Med 2020;8:2050312120934839. DOI: 10.1177/2050312120934839.
34. Normand R, Yanai I. An introduction to high-throughput sequencing experiments: Design and bioinformatics analysis. Methods Mol Biol 2013;1038:1–26. DOI: 10.1007/978-1-62703-514-9_1.
35. Lataretu M, Krautwurst S, Huska MR, et al. Targeted decontamination of sequencing data with CLEAN. NAR Genom Bioinform 2025;7(3):lqaf105. DOI: 10.1093/nargab/lqaf105.
36. Dida F, Yi G. Empirical evaluation of methods for de novo genome assembly. PeerJ Comput Sci 2021;7:e636. DOI: 10.7717/peerj-cs.636.
37. Manekar SC, Sathe SR. A benchmark study of k-mer counting methods for high-throughput sequencing. Gigascience 2018;7(12):giy125. DOI: 10.1093/gigascience/giy125.
38. Piccolo SR, Frampton MB. Tools and techniques for computational reproducibility. Gigascience 2016;5(1):30. DOI: 10.1186/s13742-016-0135-4.
39. Sarsani VK, Raghupathy N, Fiddes IT, et al. The genome of C57BL/6J "Eve", the mother of the laboratory mouse genome reference strain. G3 (Bethesda) 2019;9(6):1795–1805. DOI: 10.1534/g3.119.400071.
40. Shiou SR, Yu Y, Chen S, et al. Erythropoietin protects intestinal epithelial barrier function and lowers the incidence of experimental neonatal necrotizing enterocolitis. J Biol Chem 2011;286(14):12123–12132. DOI: 10.1074/jbc.M110.154625.
41. Carvalho BS, Irizarry RA. A framework for oligonucleotide microarray preprocessing. Bioinformatics 2010;26(19):2363–2367. DOI: 10.1093/bioinformatics/btq431.
42. Chan BKC. Data analysis using R programming. Adv Exp Med Biol 2018;1082:47–122. DOI: 10.1007/978-3-319-93791-5_2.
43. Kim Y, Doan BQ, Duggal P, et al. Normalization of microarray expression data using within-pedigree pool and its effect on linkage analysis. BMC Proc 2007;1 Suppl 1(Suppl 1):S152. DOI: 10.1186/1753-6561-1-s1-s152.
44. Sifakis EG, Prentza A, Koutsouris D, et al. Evaluating the effect of various background correction methods regarding noise reduction, in two-channel microarray data. Comput Biol Med 2012;42(1):19–29. DOI: 10.1016/j.compbiomed.2011.10.003.

45. Zhao Y, Wong L, Goh WWB. How to do quantile normalization correctly for gene expression data analyses. Sci Rep 2020;10(1):15534. DOI: 10.1038/s41598-020-72664-6.
46. Irizarry RA, Bolstad BM, Collin F, et al. Summaries of Affymetrix GeneChip probe level data. Nucleic Acids Res 2003;31(4):e15. DOI: 10.1093/nar/gng015.
47. Irizarry RA, Hobbs B, Collin F, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. Biostatistics 2003;4(2):249–264. DOI: 10.1093/biostatistics/4.2.249.
48. Ritchie ME, Phipson B, Wu D, et al. Limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucl Acids Res 2015;43(7):e47. DOI: 10.1093/nar/gkv007.
49. Smyth GK. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat Appl Genet Mol Biol 2004;3(1):Article3. DOI: 10.2202/1544-6115.1027.
50. Hui HWH, Kong W, Goh WWB. Thinking points for effective batch correction on biomedical data. Brief Bioinform 2024;25(6):bbae515. DOI: 10.1093/bib/bbae515.
51. Sprang M, Andrade-Navarro MA, Fontaine JF. Batch effect detection and correction in RNA-seq data using machine-learning-based automated assessment of quality. BMC Bioinformatics 2022;23(Suppl 6):279. DOI: 10.1186/s12859-022-04775-y.
52. Groth D, Hartmann S, Klie S, et al. Principal components analysis. Methods Mol Biol 2013;930:527–547. DOI: 10.1007/978-1-62703-059-5_22.
53. Guess MJ, Wilson SB. Introduction to hierarchical clustering. J Clin Neurophysiol 2002;19(2):144–151. DOI: 10.1097/00004691-200203000-00005.
54. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005;102(43):15545–15550. DOI: 10.1073/pnas.0506580102.
55. Mootha VK, Lindgren CM, Eriksson K-F, et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34(3):267–273. DOI: 10.1038/ng1180.
56. Zito A, Lualdi M, Granata P, et al. Gene set enrichment analysis of interaction networks weighted by node centrality. Front Genet 2021;12:577623. DOI: 10.3389/fgene.2021.577623.
57. Liberzon A, Birger C, Thorvaldsdottir H, et al. The molecular signatures database (MSigDB) hallmark gene set collection. Cell Syst 2015;1(6):417–425. DOI: 10.1016/j.cels.2015.12.004.
58. Harris MA, Clark J, Ireland A, et al. The gene ontology (GO) database and informatics resource. Nucleic Acids Res 2004;32(Database issue):D258–D261. DOI: 10.1093/nar/gkh036.
59. Roncaglia P, Martone ME, Hill DP, et al. The gene ontology (GO) cellular component ontology: Integration with SAO (subcellular anatomy ontology) and other recent developments. J Biomed Semantics 2013;4(1):20. DOI: 10.1186/2041-1480-4-20.
60. Godbold G, Proescher J, Gaudet P. New and revised gene ontology biological process terms describe multiorganism interactions critical for understanding microbial pathogenesis and sequences of concern. J Biomed Semantics 2025 21;16(1):4. DOI: 10.1186/s13326-025-00323-8.
61. Lu Z, Hunter L. Go molecular function terms are predictive of subcellular localization. Pac Symp Biocomput 2005:151–161. DOI: 10.1142/9789812702456_0015.
62. Kanehisa M, Goto S. KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 2000;28(1):27–30. DOI: 10.1093/nar/28.1.27.
63. Milacic M, Beavers D, Conley P, et al. The reactome pathway knowledgebase 2024. Nucleic Acids Res 2024;52(D1):D672–D678. DOI: 10.1093/nar/gkad1025.
64. Glickman ME, Rao SR, Schultz MR. False discovery rate control is a recommended alternative to Bonferroni-type adjustments in health studies. J Clin Epidemiol 2014;67(8):850–857. DOI: 10.1016/j.jclinepi.2014.03.012.
65. Dessau RB, Pipper CB. ["R"--project for statistical computing]. Ugeskr Laeger 2008 28;170(5):328–330. PMID: 18252159.
66. Wu HL, Kaufman ID, Hsu PY. A ggplot-based single-gene viewer reveals insights into the translatome and other nucleotide-resolution omics data. bioRxiv 2025. DOI: 10.1101/2025.01.30.635743.
67. Wickham H, Chang W, Wickham M. Package 'ggplot2'Create Elegant Data Visualisations Using the Grammar of Graphics; Version 2. 2016;2016.
68. Maheshwari A. Severe anemia predisposes very premature infants to transfusion-associated necrotizing enterocolitis. Semin Fetal Neonatal Med 2025;30(1):101615. DOI: 10.1016/j.siny.2025.101615.
69. Pirim H, Eksioglu B, Perkins A, et al. Clustering of high throughput gene expression data. Comput Oper Res 2012;39(12):3046–3061. DOI: 10.1016/j.cor.2012.03.008.
70. Jaskowiak PA, Campello RJGB, Costa IG. On the selection of appropriate distances for gene expression data clustering. BMC Bioinformatics 2014;15 Suppl 2(Suppl 2):S2. DOI: 10.1186/1471-2105-15-S2-S2.
71. Alonso ME, Pernaute B, Crespo M, et al. Understanding the regulatory genome. Int J Dev Biol 2009;53(8–10):1367–1378. DOI: 10.1387/ijdb.072428ma.
72. Usui N, Berto S, Konishi A, et al. Zbtb16 regulates social cognitive behaviors and neocortical development. Transl Psychiatry 2021;11(1):242. DOI: 10.1038/s41398-021-01358-y.
73. Gu W, Eke C, Santiago EG, et al. Single-cell atlas of the small intestine throughout the human lifespan demonstrates unique features of fetal immune cells. Mucosal Immunol 2024;17(4):599–617. DOI: 10.1016/j.mucimm.2024.03.011.
74. Bernaudo S, Salem M, Qi X, et al. Cyclin G2 inhibits epithelial-to-mesenchymal transition by disrupting Wnt/beta-catenin signaling. Oncogene 2016;35(36):4816–4827. DOI: 10.1038/onc.2016.15.
75. McLaughlin KL, Nelson MAM, Coalson HS, et al. Bioenergetic phenotyping of DEN-induced hepatocellular carcinoma reveals a link between adenylate kinase isoform expression and reduced complex I-supported respiration. Front Oncol 2022;12:919880. DOI: 10.3389/fonc.2022.919880.
76. Wujak M, Veith C, Wu CY, et al. Adenylate kinase 4-A key regulator of proliferation and metabolic shift in human pulmonary arterial smooth muscle cells via Akt and HIF-1alpha signaling pathways. Int J Mol Sci 2021;22(19):10371. DOI: 10.3390/ijms221910371.
77. Hamel LP, Benchabane M, Nicole MC, et al. Stress-responsive mitogen-activated protein kinases interact with the EAR motif of a poplar zinc finger protein and mediate its degradation through the 26S proteasome. Plant Physiol 2011;157(3):1379–1393. DOI: 10.1104/pp.111.178343.
78. Machado ACD, Cooper BH, Lei X, et al. Landscape of DNA binding signatures of myocyte enhancer factor-2B reveals a unique interplay of base and shape readout. Nucleic Acids Res 2020;48(15):8529–8544. DOI: 10.1093/nar/gkaa642.
79. Molkentin JD, Firulli AB, Black BL, et al. MEF2B is a potent transactivator expressed in early myogenic lineages. Mol Cell Biol 1996;16(7):3814–3824. DOI: 10.1128/MCB.16.7.3814.
80. Drolet DW, Scully KM, Simmons DM, et al. TEF, a transcription factor expressed specifically in the anterior pituitary during embryogenesis, defines a new class of leucine zipper proteins. Genes Dev 1991;5(10):1739–1753. DOI: 10.1101/gad.5.10.1739.
81. Waeytens A, De Vos M, Laukens D. Evidence for a potential role of metallothioneins in inflammatory bowel diseases. Mediators Inflamm 2009;2009:729172. DOI: 10.1155/2009/729172.
82. Lee C, Kim MJ, Kumar A, et al. Vascular endothelial growth factor signaling in health and disease: From molecular mechanisms to therapeutic perspectives. Signal Transduct Target Ther 2025;10(1):170. DOI: 10.1038/s41392-025-02249-0.
83. Padilla-Vaca F, de la Mora J, Garcia-Contreras R, et al. Two-component system sensor kinases from asgardian archaea may be witnesses to eukaryotic cell evolution. Molecules 2023;28(13):5042. DOI: 10.3390/molecules28135042.
84. Matthews CC, Zielke HR, Parks DA, et al. Glutamate-pyruvate transaminase protects against glutamate toxicity in hippocampal

slices. Brain Res 2003;978(1-2):59–64. DOI: 10.1016/s0006-8993(03)02765-3.
85. Moriles KE, Zubair M, Azer SA. Alanine Aminotransferase (ALT) Test. Treasure Island, FL: StatPearls. 2025.
86. Mascarell L, Auger R, Alcover A, et al. Characterization of a gene encoding two isoforms of a mitochondrial protein up-regulated by cyclosporin A in activated T cells. J Biol Chem 2004 12;279(11):10556–10563. DOI: 10.1074/jbc.M313770200.
87. Amesse LS, Moulton R, Zhang YM, et al. Expression of HOX gene products in normal and abnormal trophoblastic tissue. Gynecol Oncol 2003;90(3):512–518. DOI: 10.1016/s0090-8258(03)00357-3.
88. Brand NJ. Myocyte enhancer factor 2 (MEF2). Int J Biochem Cell Biol 1997;29(12):1467–1470. DOI: 10.1016/s1357-2725(97)00084-8.
89. Dalmeijer GW, van der Schouw YT, Magdeleyns EJ, et al. Matrix Gla protein species and risk of cardiovascular events in type 2 diabetic patients. Diabetes Care 2013;36(11):3766–3771. DOI: 10.2337/dc13-0065.
90. Sun W, Song J, Wu Q, et al. Regulator of ribosome synthesis 1 (RRS1) stabilizes GRP78 and promotes breast cancer progression. Molecules 2024 28;29(5):1051. DOI: 10.3390/molecules29051051.
91. Sivri FNB, Ciftci S. A new insight into fatty acid binding protein 4 mechanisms and therapeutic implications in obesity-associated diseases: A mini review. Mol Nutr Food Res 2024;68(8):e2300840. DOI: 10.1002/mnfr.202300840.
92. Ryu N, Sagong B, Park HJ, et al. Screening of the SLC17A8 gene as a causative factor for autosomal dominant non-syndromic hearing loss in Koreans. BMC Med Genet 2016;17:6. DOI: 10.1186/s12881-016-0269-3.
93. Boggs JM. Myelin basic protein: A multifunctional protein. Cell Mol Life Sci 2006;63(17):1945–1961. DOI: 10.1007/s00018-006-6094-7.
94. Harms MB, Birn R, Provencal N, et al. Early life stress, FK506 binding protein 5 gene (FKBP5) methylation, and inhibition-related prefrontal function: A prospective longitudinal study. Dev Psychopathol 2017;29(5):1895–1903. DOI: 10.1017/S095457941700147X.
95. Noori M, Jarrah O, Al Shamsi A. Carbamoly-phosphate synthetase 1 (CPS1) deficiency: A tertiary center retrospective cohort study and literature review. Mol Genet Metab Rep 2024;41:101156. DOI: 10.1016/j.ymgmr.2024.101156.
96. Wang Y, Xu X, Marshall JE, et al. Loss of hyaluronan and proteoglycan link protein-1 induces tumorigenesis in colorectal cancer. Front Oncol 2021;11:754240. DOI: 10.3389/fonc.2021.754240.
97. Porras AM, Zhou H, Shi Q, et al. Inflammatory bowel disease-associated gut commensals degrade components of the extracellular matrix. mBio 2022;13(6):e0220122. DOI: 10.1128/mbio.02201-22.
98. Sollberger G, Strittmatter GE, Garstkiewicz M, et al. Caspase-1: The inflammasome and beyond. Innate Immun 2014;20(2):115–125. DOI: 10.1177/1753425913484374.
99. Ali I, Yang WC. The functions of kinesin and kinesin-related proteins in eukaryotes. Cell Adh Migr 2020;14(1):139–152. DOI: 10.1080/19336918.2020.1810939.
100. Sheng L, Hao SL, Yang WX, et al. The multiple functions of kinesin-4 family motor protein KIF4 and its clinical potential. Gene 2018;678:90–99. DOI: 10.1016/j.gene.2018.08.005.
101. Silva JPN, Silva PMA, Bousbaa H. Kinesin spindle protein (KIF11) in mitosis and cancer. Int J Mol Sci 2025;26(18):8975. DOI: 10.3390/ijms26188975.
102. Sun X, Kaufman PD. Ki-67: More than a proliferation marker. Chromosoma 2018;127(2):175–186. DOI: 10.1007/s00412-018-0659-8.
103. Liu T, Gu Y, Zhang Y, et al. Integrin alpha2 in the microenvironment and the tumor compartment of digestive (gastrointestinal) cancers: Emerging regulators and therapeutic opportunities. Front Oncol 2024;14:1439709. DOI: 10.3389/fonc.2024.1439709.
104. Stevenson BW, Gorman MA, Koach J, et al. A structural view of PA2G4 isoforms with opposing functions in cancer. J Biol Chem 2020;295(47):16100–16112. DOI: 10.1074/jbc.REV120.014293.
105. Engstrom Y, Rozell B, Hansson HA, et al. Localization of ribonucleotide reductase in mammalian cells. EMBO J 1984;3(4):863–867. DOI: 10.1002/j.1460-2075.1984.tb01897.x.
106. Lan P, Zhou B, Tan M, et al. Structural insight into precursor ribosomal RNA processing by ribonuclease MRP. Science 2020;369(6504):656–663. DOI: 10.1126/science.abc0149.
107. Jiang Q, Chen Q, Sun Q, et al. NADPH oxidase activator 1 (NOXA1) suppresses ferroptosis and radiosensitization in colorectal cancer. Int J Med Sci 2025;22(6):1301–1312. DOI: 10.7150/ijms.107038.
108. Stenke E, Aviello G, Singh A, et al. NADPH oxidase 4 is protective and not fibrogenic in intestinal inflammation. Redox Biol 2020;37:101752. DOI: 10.1016/j.redox.2020.101752.
109. Jarde T, Chan WH, Rossello FJ, et al. Mesenchymal niche-derived neuregulin-1 drives intestinal stem cell proliferation and regeneration of damaged epithelium. Cell Stem Cell 2020;27(4):646–662.e7. DOI: 10.1016/j.stem.2020.06.021.
110. Hernandez JE, Llorente C, Ma S, et al. The arginine methyltransferase PRMT5 promotes mucosal defense in the intestine. Life Sci Alliance 2023;6(11):e202302026. DOI: 10.26508/lsa.202302026.
111. Chen DY, Zhang YY, Nie HH, et al. Comprehensive analyses of solute carrier family members identify SLC12A2 as a novel therapy target for colorectal cancer. Sci Rep 2024;14(1):4459. DOI: 10.1038/s41598-024-55048-y.
112. Marrone A, Walne A, Dokal I. Dyskeratosis congenita: Telomerase, telomeres and anticipation. Curr Opin Genet Dev 2005;15(3):249–257. DOI: 10.1016/j.gde.2005.04.004.
113. Lin SZ, Wu WJ, Cheng YQ, et al. Prolyl endopeptidase remodels macrophage function as a novel transcriptional coregulator and inhibits fibrosis. Exp Mol Med 2023;55(7):1437–1450. DOI: 10.1038/s12276-023-01027-8.
114. Pitarch B, Chagoyen M, Ranea JAG, et al. A review on gene ontology evaluations. Database (Oxford) 2025;2025:baaf058. DOI: 10.1093/database/baaf058.
115. Parikh JR, Klinger B, Xia Y, et al. Discovering causal signaling pathways through gene-expression patterns. Nucleic Acids Res 2010;38(Web Server issue):W109–W117. DOI: 10.1093/nar/gkq424.
116. Tian L, Greenberg SA, Kong SW, et al. Discovering statistically significant pathways in expression profiling studies. Proc Natl Acad Sci U S A 2005 20;102(38):13544–13549. DOI: 10.1073/pnas.0506577102.
117. Harbig J, Sprinkle R, Enkemann SA. A sequence-based identification of the genes detected by probesets on the Affymetrix U133 plus 2.0 array. Nucleic Acids Res 2005;33(3):e31. DOI: 10.1093/nar/gni027.
118. Hardison RC. Evolution of hemoglobin and its genes. Cold Spring Harb Perspect Med 2012;2(12):a011627. DOI: 10.1101/cshperspect.a011627.
119. Maheshwari A. The phylogeny, ontogeny, and organ-specific differentiation of macrophages in the developing intestine. Newborn (Clarksville) 2022;1(4):340–355. DOI: 10.5005/jp-journals-11002-0044.
120. Osakada T, Abe T, Itakura T, et al. Hemoglobin in the blood acts as a chemosensory signal via the mouse vomeronasal system. Nat Commun 2022;13(1):556. DOI: 10.1038/s41467-022-28118-w.
121. Bard H, Prosmanne J. Postnatal fetal and adult hemoglobin synthesis is preterm infants whose birth weight was less than 1,000 grams. J Clin Invest 1982;70(1):50–52. DOI: 10.1172/jci110602.
122. Still much to learn about mice. Nature 2014;509(7501):399. DOI: 10.1038/509399a.
123. Osier ND, Pham L, Savarese A, et al. Animal models in genomic research: Techniques, applications, and roles for nurses. Appl Nurs Res 2016;32:247–256. DOI: 10.1016/j.apnr.2016.07.016.
124. Ho TTB, Groer MW, Kane B, et al. Dichotomous development of the gut microbiome in preterm infants. Microbiome 2018;6(1):157. DOI: 10.1186/s40168-018-0547-8.
125. Ho TBT, Groer MW, Kane B, et al. Enteric dysbiosis and fecal calprotectin expression in premature infants. Pediatr Res 2019;85(3):361–368. DOI: 10.1038/s41390-018-0254-y.

126. Sankaran VG, Orkin SH. The switch from fetal to adult hemoglobin. Cold Spring Harb Perspect Med 2013;3(1):a011643. DOI: 10.1101/cshperspect.a011643.
127. Pannucci CJ, Wilkins EG. Identifying and avoiding bias in research. Plast Reconstr Surg 2010;126(2):619–625. DOI: 10.1097/PRS.0b013e3181de24bc.
128. Chen W, Dumoulin A, Li X, et al. Transposing sequences between fetal and adult hemoglobins indicates which subunits and regulatory molecule interfaces are functionally related. Biochemistry 2000;39(13):3774–3781. DOI: 10.1021/bi992691l.
129. Nie X, Guang P, Peng X. Critical components for designing and implementing randomized controlled trials. Pediatr Investig 2018;2(2):124–130. DOI: 10.1002/ped4.12042.
130. Giraudeau B, Caille A, Eldridge SM, et al. Heterogeneity in pragmatic randomised trials: Sources and management. BMC Med 2022;20(1):372. DOI: 10.1186/s12916-022-02569-w.

CLINICAL TECHNIQUE

Clinical Procedures: Use of Peripherally Inserted Central Catheters in Neonates

Monika Kaushal[1], Kalyan C Balla[2]

Received on: 06 October 2025; *Accepted on*: 11 December 2025; *Published on*: 15 January 2026

Abstract

Peripherally inserted central catheters (PICCs) are widely used in neonatal intensive care units (NICUs) to provide durable central venous access in premature/critically ill infants. Point-of-care ultrasound (POCUS)-guided PICC insertion has improved the first-pass success rates of insertion of PICC lines, reducing complications and minimizing radiation exposure from repeated X-rays. This article outlines an evidence-based, standardized approach to PICC insertion, care, and verification in neonates.

Keywords: Basilic, Brachial, Cavoatrial junction, Cephalic, Coagulopathy, Cyanoacrylate glue, Great saphenous vein, High-frequency linear probe, Infant, Median cubital vein, Micro-Seldinger technique, Newborn, Peripherally inserted central catheter, Point-of-care ultrasound, Polyurethane, Radiation exposure, Silicone, Vesicant medications.

Newborn (2025): 10.5005/jp-journals-11002-0143

Key Points

- Peripherally inserted central catheters (PICCs) are widely used in neonatal intensive care units (NICUs) to provide durable central venous access in premature/critically ill infants;
- Neonatal PICC lines are soft, flexible tubes typically made from biocompatible materials like polyurethane or silicone;
- Guidance with point-of-care ultrasound (POCUS) for PICC insertion has improved first-pass success rates, reduced complications, and minimized radiation exposure from repeated X-rays;
- This article has summarized the indications, techniques, and the role of US guidance in the insertion of PICCs in neonates.

Introduction

Peripherally inserted central catheters are widely used in NICUs to provide secure and durable central venous access for parenteral nutrition, medications, and fluids. Neonatal PICC lines are soft, flexible tubes typically made from biocompatible materials like polyurethane or silicone. Point-of-care ultrasound-guided PICC insertion has emerged as a superior technique, improving first-pass success rates, reducing complications, and minimizing radiation exposure from repeated X-rays.[1–7] Simulation-based training for NICU staff significantly improves insertion accuracy and procedural confidence, translating into higher first-pass success and fewer malpositions.[5]

This protocol outlines an evidence-based, standardized approach to PICC insertion, care, and verification in neonates (Fig. 1).

Indications

Peripherally inserted central catheter placement is recommended in neonates who require the following:[1,2]

- Intravenous fluids >14 days;
- Delivery of irritant or vesicant medications;
- Parenteral nutrition;
- Power-injectable access;
- Frequent or reliable blood sampling;
- Clinically unstable infants requiring secure central access; and
- Neonates with difficult peripheral venous access.

Contraindications

- Local infection at the insertion site;
- Severe coagulopathy (without correction);
- Known thrombosis in the target vessel;
- Insufficient vessel diameter (<2 mm);
- Severe edema or limb injury at the planned insertion site.

Vein Selection

Preferred Veins

Upper Limb: Basilic vein (first choice), cephalic vein, brachial vein;

Lower Limb: Great saphenous vein, femoral vein (with caution);

Superficial veins [for extended Dwell catheter (EDC), not PICC]: Median cubital vein, superficial temporal vein.

Evidence-based Considerations

Upper limb veins have higher first-pass success and lower thrombosis rates; lower limb veins may be used when upper limb

[1]Department of Neonatology, Emirates Specialty Hospital, Dubai Healthcare City, Dubai, United Arab Emirates

[2]Department of Neonatology, Emirates Hospital, Jumeirah, United Arab Emirates

Corresponding Author: Monika Kaushal, Department of Neonatology, Emirates Specialty Hospital, Dubai Healthcare City, Dubai, United Arab Emirates, e-mail: monikakaushal022@gmail.com

How to cite this article: Kaushal M, Balla KC. Clinical Procedures: Use of Peripherally Inserted Central Catheters in Neonates. Newborn 2025;4(4):178–180.

Source of support: Nil

Conflict of interest: None

access is not feasible.[2,3] Preprocedure US assessment ensures vessel patency and diameter suitability (>2 mm).[1,3]

Success Rates

Upper limb overall success: ~99%; lower limb success: ~70%.[2,3]

Catheter Selection

- Catheter size must not exceed the internal diameter of the vein to reduce thrombosis risk;
- Typical neonatal PICC size: 1–2.8 Fr;
- Use power-injectable catheters if required;[1,2]
- Avoid double-lumen PICCs in neonates;
- Extended dwell catheter or midline catheters may be considered for short-term therapy (<14 days) or superficial veins.

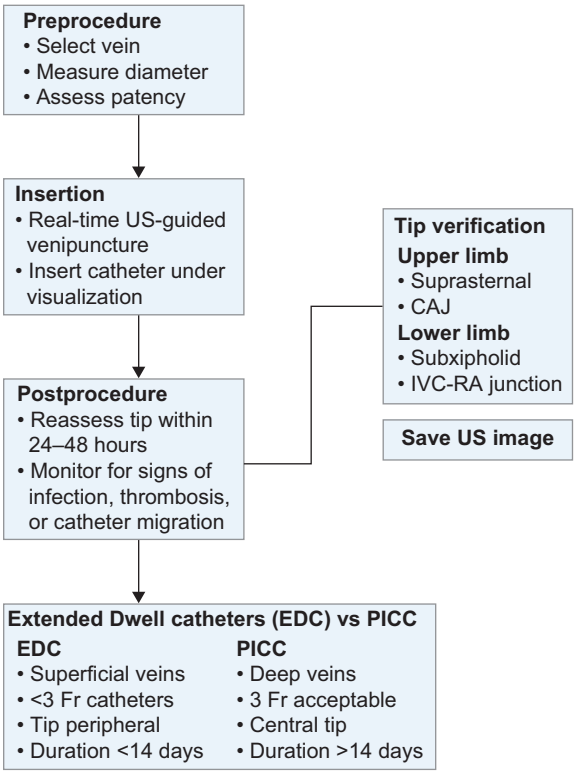


Fig. 1: Neonatal PICC protocol

CAJ, cavoatrial junction; Fr, French gauge; US, ultrasound

Ultrasound (POCUS) Guidance

Equipment

High-frequency linear probe 10–18 MHz is useful. A sterile sheath and gel and a 24-G or smaller introducer needle are used. A micro-Seldinger technique (MST) set can be used when available.

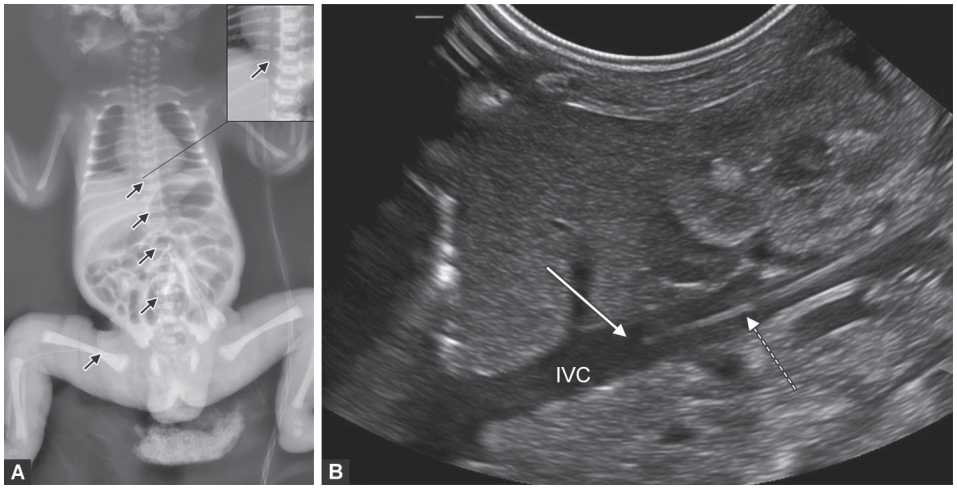
Technique

- Position the infant and secure the extremity;
- Scan the vein in short axis (transverse) to identify patency, depth, and compressibility;
- Confirm the diameter as >2 mm, no thrombi, and adequate depth (>7 mm);[1–3]
- Perform venipuncture under direct US visualization (out-of-plane or in-plane technique);
- Insert guidewire (if MST used), then advance catheter while continuously tracking the tip under US guidance;[1,2,4]
- Simulation-based practice improves operator skill, reduces malposition, and increases success rates.[5]

Tip Position Verification

Point-of-care ultrasound for tip confirmation is reliable, rapid, and reduces radiation exposure:[2,3,6,7]

- For upper limb PICCs, we usually target the SVC–RA junction. The recommended view is the suprasternal long-axis, vertebral range of T2–T10.
- To confirm the placement of lower limb PICCs (Fig. 2), we typically target the IVC–RA junction to locate the catheter in the subcostal/subxiphoid views at the T8–T10 vertebral level. We usually save US clips or still images for follow-up comparisons.
- Real-time assessment allows correction before catheter use, decreases malposition risk, and may reduce X-ray dependence.[1,2,7]



Figs 2A and B: Lower limb PICCs. (A) We typically seek to place the catheter tip at the inferior vena cava-right atrial junction, which is usually seen at the T8–T10 vertebral level in subcostal/subxiphoid views. (B) Ultrasound images are often similar/more helpful in locating the catheter tip, with the added advantage of achieving these goals without radiation exposure

Cyanoacrylate Glue (Securement)

- Recommended as part of the central line bundle;
- Reduces catheter dislodgement, infection risk, and mechanical complications;
- Safe in neonates with no reported skin irritation.[1,4]

Postinsertion Care

- We inspect the site every shift;
- Use a transparent semipermeable dressing;
- Flush with heparinized saline per unit protocol;
- Daily line necessity review;
- Ultrasound reassessment if limb movement or concern for malposition;[1,3]
- Maintain strict aseptic technique during dressing changes and access.

Complications and Prevention

- Malpositions can be checked/minimized with real-time US.
- Arterial punctures can be minimized with short-axis guidance.
- To reduce the risk of thrombosis, we avoid oversizing catheters and ensure that the tip is central.
- To minimize infections, we use cyanoacrylate, sterile barrier precautions, and review the insertion sites and catheter flow rates daily.
- To reduce the risk of phlebitis, we choose the upper limb access whenever possible.
- Periodic POCUS assessment helps in early recognition of complications.[1–3,6,7]

PICC Algorithm (Summary)

- Preprocedure: Select vein → measure diameter → assess patency → check contraindications.
- Insertion: Real-time US-guided venipuncture → insert catheter under visualization, monitor guidewire, and catheter advancement via US.
- Tip verification: (a) upper limb: Suprasternal → cavoatrial junction (CAJ); (b) lower limb: Subxiphoid → IVC–RA junction. We save US images for documentation.
- Post-procedure: We reassess the position of the tip within 24–48 hours and monitor for signs of infection, thrombosis, or catheter migration.

EDC vs PICC

Feature	*ECC (Midline catheter)*	*PICC*
Vein	Superficial veins	Deeper veins (>7 mm)
Catheter size	<3 Fr	3 Fr acceptable
Sampling	Not suited	Suitable for sampling
Duration	<14 days	>14 days
Tip location	Not central	Central tip

Orcid

Monika Kaushal https://orcid.org/0000-0002-4866-6288

References

1. Fridolfsson PEJ. Ultrasound-guided peripherally inserted central catheter placement in extremely low birth weight neonates. Neonatal Netw 2022;41(1):21–37. DOI: 10.1891/11-T-733.
2. Grasso F, Capasso A, Pacella D, et al. Ultrasound guided catheter tip location in neonates: A prospective cohort study. J Pediatr 2022;244:86–91.e2. DOI: 10.1016/j.jpeds.2021.12.059.
3. Firszt O, Maślanka M, Grabowska A, et al. Standardized ultrasound protocol for peripherally inserted central catheters in neonates: A retrospective, X-ray controlled observational study. Children (Basel) 2024;11(10):1204. DOI: 10.3390/children11101204.
4. Meinen RD, Bauer AS, Devous K, et al. Point-of-care ultrasound use in umbilical line placement: A review. J Perinatol 2020;40(4):560–566. DOI: 10.1038/s41372-019-0558-8.
5. Andreatta P, Chen Y, Marsh M, et al. Simulation-based training improves applied clinical placement of ultrasound-guided PICCs. Support Care Cancer 2011;19(4):539–543. DOI: 10.1007/s00520-010-0849-2.
6. Singh Y, Tissot C, Fraga MV, et al. International evidence-based guidelines on point of care ultrasound (POCUS) for critically ill neonates and children issued by the POCUS Working Group of the European Society of Paediatric and Neonatal Intensive Care (ESPNIC). Crit Care 2020;24(1):65. DOI: 10.1186/s13054-020-2787-9.
7. Doyle SC, Bergin NM, Young R, et al. Diagnostic accuracy of ultrasound for localising peripherally inserted central catheter tips in infants in the neonatal intensive care unit: A systematic review and meta-analysis. Pediatr Radiol 2022;52(12):2421–2430. DOI: 10.1007/s00247-022-05379-7.

CLINICAL TECHNIQUE

Systematized Systemic Sonographic Surveillance (S4)

Srijan Singh[1–3], Monika Kaushal[2,4], Brunetta Guaragni[2,5], Salvatore Aversa[2,5], Naief Alghnimei[2,6], Md Rezaul Hayat[7,8], Ayush Kaushal[9], Ghania Daar Ede[2,10], Jeremías PGB Duré[2,11], Jargalsaikhan Badarch[2,12], Md Mozibur Rahman[2,13–15], Ola D Saugstad[2,14,15], Rachana Singh[2,16], Akhil Maheshwari[2,3,12,13,15,17,19–30]

Received on: 15 October 2025; *Accepted on*: 08 December 2025; *Published on*: 15 January 2026

Abstract

We are developing a systematized systemic sonographic surveillance (S4) program as a structured, sonographic technology-enabled extension of the physical examination of newborn infants. The philosophy of S4 differs from point-of-care ultrasound (POCUS), which is known for *post hoc* bedside confirmation/assessment/temporal monitoring of organ-system injury in at-risk/critically-ill patients. Systematized systemic sonographic surveillance utilizes radiation-free, real-time imaging for *a priori* assessment of risk or detection of sub-clinical lesions. We examine the brain (growth, hemorrhages), heart (preload, contractility), lungs (edema, atelectasis, consolidation, effusions, and pneumothorax), liver (size, parenchymal abnormalities, vascular complications), bowel (edema, contractility, ascites, early detection of sub-clinical necrotizing enterocolitis), urogenital tract, spine, and hips (developmental dysplasia). Visual guidance can also help in the placement of central lines and successful atraumatic performance of lumbar punctures (LPs). Systematized systemic sonographic surveillance can also reduce the duration of training and the need for sedation and transport for the evaluation of patients. Increased efficiency in clinical procedures may reduce the duration and intensity of pain and, consequently, have a positive impact on development. To summarize, sonographic surveillance could improve clinical decision-making and neonatal intensive care unit (NICU) outcomes. The costs of infrastructural enhancements in sonography still need evaluation vis-à-vis potential savings from reduced needs for disposables, short- and long-term morbidity, and the length of hospital stay.

Keywords: A-lines, B-lines, Collapsibility index, Developmental dysplasia of the hip, Distensibility index, Dynamic LP, Endotracheal tube tip position, Graf classification, Hemodynamics, Hepatization, Intraventricular hemorrhage, Lung ultrasound, Lung ultrasound score, Multi-organ sonography, Neonatal intensive care unit, Neonate, Percutaneous central catheters, Point-of-care ultrasound, Screening, Shred sign, Static LP, Systematized systemic sono-surveillance, Training barriers, Umbilical arterial line, Umbilical venous line, Vascular access guidance, α-angle, β-angle.

Newborn (2025): 10.5005/jp-journals-11002-0142

Key Points

- We are developing a systematized systemic sonographic surveillance (S4) program as a structured, comprehensive sonographic technology-enabled extension of the physical examination of newborn infants.
- Systematized systemic sonographic surveillance can enable *a priori* monitoring of the risk or help in detecting sub-clinical lesions involving the brain/lungs/kidneys/gut/genitalia/joints. In procedures such as placement of central lines and lumbar punctures (LPs), S4 can improve efficiency and reduce the need for transport to higher-level neonatal units.
- The philosophy of S4 contrasts with that of point-of-care ultrasound (POCUS), which is directed at *post hoc* confirmation/assessment/monitoring of organ-system injury.
- We believe that S4, by providing visual guidance, enhances the efficiency/efficacy of procedures and shortens the duration of clinical training. Reducing the number of attempts needed to accomplish these procedures will reduce the cumulative inflicted pain and thereby have a positive impact on development. These impressions need to be carefully tested.
- In the longer term, sonographic surveillance may provide more clinical information and thereby improve medical decision-making and neonatal intensive care unit (NICU) outcomes.
- The costs of these infrastructural enhancements will need to be evaluated vis-à-vis potential savings from reduced needs for disposables, morbidity, and length of hospital stay.

[1]Department of Neonatology, Yashoda Medicity, Indirapuram, Uttar Pradesh, India

[2]Global Newborn Society, Harrison, New York, United States of America

[3]GNS Forum for Transgenerational Inheritance, New York, United States of America

[4]Department of Neonatology, Emirates Specialty Hospital Dubai Healthcare City Dubai, United Arab Emirates

[5]Department of Neonatology, Children's Hospital ASST Spedali Civili of Brescia, Brescia, Italy

[6]Department of Orthopedic Surgery, King Salman Armed Forces Hospital, Tabuk, Saudi Arabia

[7]Department of Paediatric Cardiology, Ministry of Health and Family Welfare, Dhaka, Bangladesh

[8]Department of Neonatal/Paediatric Cardiology, Bangladesh Neonatal Hospital, Dhaka, Bangladesh

[9]Department of Pediatrics, Aarupadai Veedu Medical College & Hospital, Kirumampakkam, Puducherry, India

[10]Department of Neonatology/Pediatrics, Sidra Medicine, Doha, Qatar

[11]Department of Neonatology, Centro Médico Nacional- Hospital Nacional de Itaugua, Paraguay

[12]Mongolian Association of Obstetrics, Gynecology, and Neonatology, UlaanBaatar, Mongolia

[13]Department of Neonatology, Institute of Maternal and Child Health, Matuil, Dhaka, Bangladesh

[14]Bangladesh Neonatal Foundation, Dhaka, Bangladesh

Introduction

We are beginning to use hand-held ultrasound for a comprehensive extension of the bedside physical examination in infants; this protocol is described as systematized systemic sonographic surveillance (S4).[1] The concept differs from POCUS, which was designed for *post hoc* sonographic confirmation/assessment/temporal evolution of organ-system injury in at-risk patients.[2–7] The key difference is in timing: S4 is viewed as a technology-enabled *a priori* evaluation of health/development/sub-clinical pathology in various organs: Brain [structural maturation and minor intraventricular hemorrhages (IVHs)], heart (preload, volumes, and contractility to measure hydration and cardiac function), lungs (maturation and sub-clinical atelectasis, consolidation, effusion(s), and air leaks), liver (growth, vascular complications and parenchymal abnormalities), intestine (contractility, bowel wall edema, ascites, early detection of sub-clinical necrotizing enterocolitis), urogenital tract, spine, and hips [maturation, developmental dysplasia of the hip (DDH)].[8–21] The radiation-free, real-time imaging in S4 can extend our abilities in detection/monitoring of sub-clinical lesions.[5]

The visual guidance in S4 can improve efficiency/efficacy/accuracy of "blind" procedures such as placement of central lines, endotracheal tubes (ETTs), and LPs; this can improve training and reduce the need for sedation and transport just for evaluation.[22–28] Increased efficiency could also reduce pain in neonatal care; fewer attempts and less need for analgesics to accomplish painful procedures might have a positive impact on development. To summarize, sonographic surveillance could improve clinical decision-making and NICU outcomes.[5] The costs of infrastructural enhancement in sonography need evaluation vis-à-vis potential savings from reduced needs for disposables, short- and long-term morbidity, and length of hospital stay.[5,7,29,30]

To emphasize again, the major attribute of S4 is in timing; it is a proactive, serial surveillance of key organ systems to monitor growth, detect early pathophysiological changes, guide interventions, and follow responses. Point-of-care ultrasound focuses on symptom-driven assessments to answer immediate clinical needs, such as the intravascular fluid status in a patient with septic shock.[2–6] Otherwise, S4 and POCUS protocols both emphasize clinician-performed imaging that can promote and increase the accuracy of treatment decisions.[7,31]

The infrastructural needs of S4 are similar to POCUS; we need portable, high-frequency transducers (>10 MHz linear probes) for rapidly-performed bedside scans by non-radiology and non-cardiology practitioners. The quality of images needs to be adequate for identification of key structures such as pleural lines, inferior vena cava (IVC), and free fluid in the pleural cavity/ascites, which can guide procedures safely (vascular access, LPs) and assess dynamic function (cardiac contractility, lung sliding). However, some artifacts/noise in the images may be acceptable as these serial assessments focus on function, not structure. The utilization of S4 may depend more on portability, ease of using the equipment, time needed for assessment, consistency of measured indices, and the costs perceived as acceptable by healthcare providers/institutions in the context of available financial support.

Table 1 and Figure 1 show the components of S4.

Need for S4

Timely imaging by bedside clinicians can help identify a pathophysiological state and ensure appropriate intervention(s).[32] Integration of ultrasonographic information in the physical examination can help adjust pre-test probabilities for a wide variety of conditions.[31,33–35] A wide range of NICU practitioners can be trained in S4, including subspecialty residents, pediatric residents, other medical house staff, nurses, nurse practitioners, and respiratory therapists.[32,35–37] Increasing evidence has shown that sonography could adjunctively improve infant outcomes, individualize physiology-based recommendations for care, and save lives in many clinical situations.[32,36–38]

The 2020 ESPNIC guidelines provided the first international evidence-based framework for clinician-performed sonography in neonatal and pediatric intensive care, developed through systematic review and Delphi consensus by a multidisciplinary expert panel.[31] The stratified use depended on mandated structured training and competency levels (basic to advanced), available supervision, and quality assurance. The emphasis has been on real-time clinician-performed imaging to guide urgent interventions, not to replace formal expertise in radiology.[31] These guidelines directly addressed safety concerns raised by the

[15]Dr Mozib Newborn Foundation, Dhaka, Bangladesh

[16]Department of Pediatrics, University of Oslo, Oslo, Norway

[17]Pioneers - Looking for Sustainable Ways to Reduce Infant Mortality, Oslo, Norway

[18]Department of Neonatology/Pediatrics, Tufts University School of Medicine, Boston, Massachusetts, United States of America

[19]Department of Pediatrics/Neonatology, Boston Children's Health Physicians Group at the Maria Fareri Children's Hospital, New York Medical College, Valhalla, New York, United States of America

[20]Banaras Hindu University Institute of Excellence, Varanasi, Uttar Pradesh, India

[21]S.A.B.R.E.E. Enrichment Academy, Saint Louis, Missouri, United States of America

[22]The Skylar Project, Daphne, Alabama, United States of America

[23]International Society for Marginalized Lives, Harrison, New York, United States of America

[24]PreemieWorld Foundation, Springfield, Virginia, United States of America

[25]Carlo GNS Center for Saving Lives at Birth, Birmingham, Alabama, United States of America

[26]Autism Care Network Foundation, India

[27]Neonatology-Certified Foundation, Brooksville, Texas, United States of America

[28]GNS Infant Nutrition Education Program, Harrison, New York, United States of America

[29]International Prader-Willi Syndrome Organization, Cambridge, United Kingdom

[30]First Breath of Life, Shreveport, Louisiana, United States of America

Corresponding Author: Akhil Maheshwari, Global Newborn Society, Harrison, New York, United States of America, Phone: +17089108729, e-mail: akhil@globalnewbornsociety.org

How to cite this article: Singh S, Kaushal M, Guaragni B, *et al*. Systematized Systemic Sonographic Surveillance (S4). Newborn 2025;4(4):181–193.

Source of support: Nil

Conflict of interest: Dr Md Mozibur Rahman, Dr Rachana Singh and Dr Akhil Maheshwari are associated as the Editorial Board Members of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of these Editorial Board Members and their research group.

Table 1: Systematized systemic sonographic surveillance (S4)

S. No.	*Key components of S4*	*Purpose*
1.	Head	Screening for IVHs
2.	Cardiac function	IVC/RA volume, LV contractility
3.	Lungs	Atelectasis, air leak, pleural effusion, pneumonia
4.	Liver	UVC position, liver size, biliary ducts
5.	Bowel USG	Assess peristalsis, ascites, and bowel wall thickness/perfusion. Findings suggestive of necrotizing enterocolitis should be confirmed with radiologists
6.	Kidneys, bladder, pelvis, and umbilical region	Measure kidney size, ureter/bladder size, perform renal doppler in infants with antenatal suspicion/ family history of renal anomalies, oliguria, urinary tract infections, and suspicion of renal vein thrombosis. Studies are needed to detect bladder size, especially in males, bladder volume, obstruction, and allantoic diverticulum. Usually done 48–72 hours after birth to avoid dehydration artifacts
7.	Genitalia	To detect undescended testes, hydrocele, torsion, and inguinal hernias
8.	Spine	To detect tethered cord, dermal sinus, during LP (to locate L5/S1) in neonates with lumbosacral skin markers or neurologic signs
9.	DDHs	Dynamic and static assessment of hip stability and acetabular development can be useful during the neonatal period before clear ossification centers can be seen. Graf method (static) and dynamic stress maneuvers are used: α-angle (>60° normal, <50° abnormal) and β-angle (<55° normal, >77° abnormal) are used for classifying hip morphology
10.	Lines and ETT position	Confirm position of umbilical lines, PICC, ETT

DDHs, developmental dysplasia of the hips; ETT, endotracheal tube; IVC, inferior vena cava; IVH, intraventricular hemorrhage; LP, lumbar puncture; PICC, percutaneous central catheters; RA, right atrium; USG, ultrasonography; UVC, umbilical venous catheter

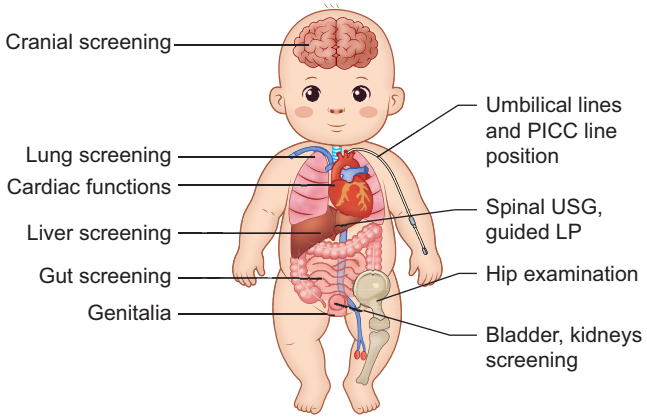


Fig. 1: Components of S4

Emergency Care Research Institute (ECRI) in its 2020 Top 10 Health Technology Hazards report, related to rapid adoption of these methods that outpaced training, experience, and skill safeguards. There were concerns about the possibility of adverse patient outcomes related to mis/underuse.[39,40] The S4 protocol aligns seamlessly with these evidence-based safeguards and extends these to anticipatory monitoring.

Components of S4

Systemic sonography can be performed in a systematic fashion to analyze the key organ systems (Fig. 1).

Head Ultrasonography (USG)

Systematized systemic sonographic surveillance begins with anterior fontanelle views (coronal and sagittal) to screen for the development of gyri/sulci and IVH. For IVH, the examiners use a phased-array or micro-convex probe to look for echogenic material in the ventricles. Serial scans (postnatal days 3–7 and weekly) are used to monitor for post-hemorrhagic hydrocephalus via ventricular index measurements. Evidence supports its superiority over clinical examination alone, reducing undetected IVH by up to 30% in NICUs. Grade III and grade IV hemorrhages can lead to neurological sequelae and disability. Table 2 shows Volpe's classification of IVH. Figure 2 shows a cranial ultrasound of a neonate with grade III IVH.

Cardiac Functions

A rapid parasternal long-axis, apical 4-chamber, and subcostal views evaluate biventricular contractility, IVC (Fig. 3) collapsibility (>50% suggests hypovolemia), and aortic coarctation. Table 3 lists the measured key parameters. Subcostal views can be used to calculate the intraventricular septal collapsibility index (CI).

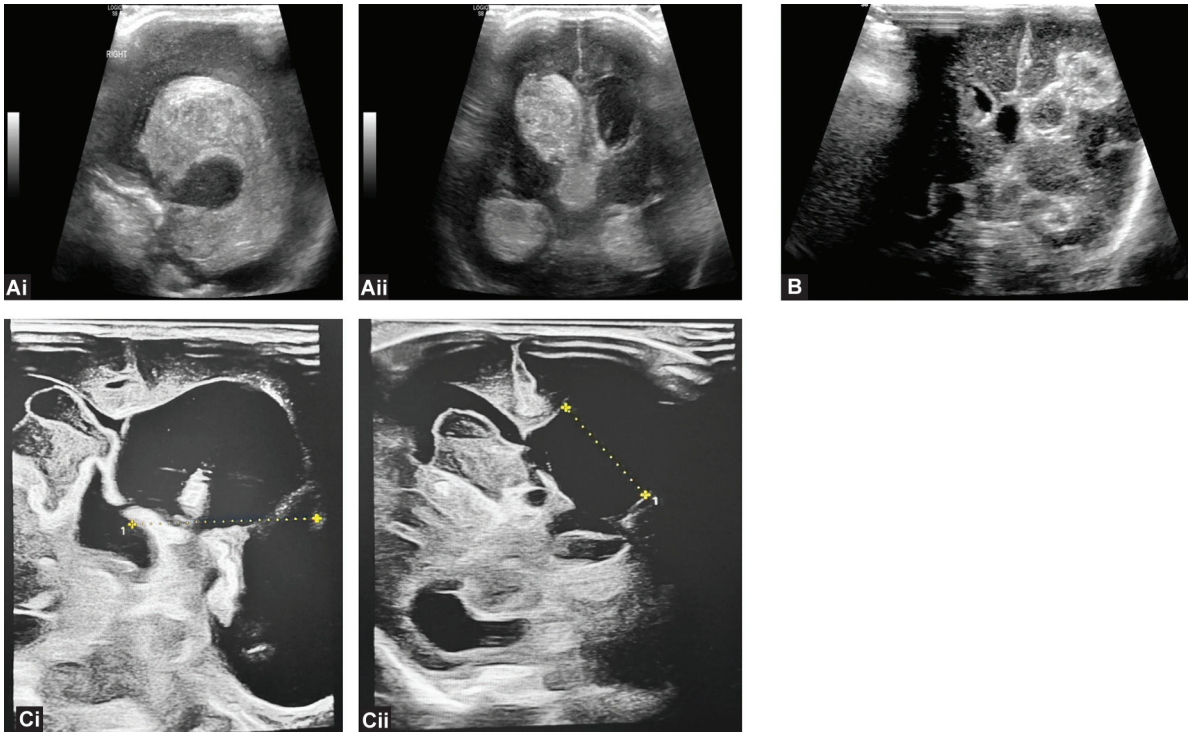
Lungs

For neonatal lung USG, a high-frequency linear array transducer probe with a frequency greater than 10 MHz is suitable for

Table 2: Volpe's classification of IVH. Grading of the severity of germinal matrix–IVH by ultrasound scan

Severity	*Description*
Grade I	Germinal matrix hemorrhage with no or minimal IVH (<10% of ventricular area on parasagittal view)
Grade II	IVH seen in 10–50% of the ventricular area on the parasagittal view
Grade III	IVH seen in >50% of the ventricular area on the parasagittal view; it usually distends the lateral ventricle
Grade IV	Notable one or more periventricular echodensities (location and extent)

IVH, intraventricular hemorrhage



Figs 2A to C: Cranial ultrasound [(Ai) Sagittal; (Aii) Coronal view)] showing grade III IVH on the left side; the ventricles are dilated by >50% (arrow); (B) Grade IV hemorrhage from the lateral ventricle into the adjacent brain parenchyma (arrow); (Ci-ii) Post-hemorrhagic hydrocephalus with moderate-severe ventricular enlargement

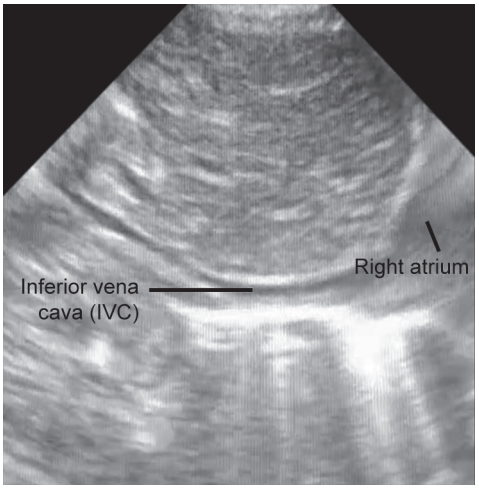


Fig. 3: Subcostal view showing the IVC entering the right atrium

examining each lung in six zones.[41–43] Higher frequencies are useful in smaller infants.

- The transducer is held perpendicular to the ribs for imaging.
- In extremely low-birthweight (ELBW) and ventilated infants, scanning the posterior lung fields can be challenging; a six-region approach can be useful where anterior upper, lower, and lateral areas of each hemithorax are visualized. Table 4 shows the scoring of lung ultrasound. Table 5 shows the scoring system for pneumonia by lung ultrasound. Figure 4 shows a lung ultrasound of a neonate with M-mode showing the "bar code sign" of pneumothorax.

Conditions such as pneumonia, acute pulmonary hemorrhage, or severe pulmonary edema cause consolidation of parenchymal segments in the lung.[44] Lung sonography can identify small pleural effusions in dependent areas, seen as anechoic spaces between the parietal and visceral pleura. In infectious conditions, the fluid could be granular, fibrinous, septated, or loculated.[7,32,35]

Table 3: Normal cardiac sonography

Parameter	*Technique/View*	*Normal values*	*Interpretation*	*Notes/Pitfalls*
IVC diameter	Subxiphoid long-axis; measure 0.5–1 cm below RA–IVC junction	3–5 mm (term) 2–3 mm (preterm)	Reflects preload; small = low volume, large = overload	Avoid probe pressure; average 3–5 cycles
CI (Spontaneous breathing)	CI = (IVCexp – IVCinsp)/ IVCexp × 100%	30–50%	↑ CI (>50%) → hypovolemia; ↓ CI (<20%) → overload/high RA pressure	Not valid if ventilated
Distensibility index (DI) (Mechanical ventilation)	DI = (IVCinsp – IVCexp)/ IVCexp × 100%	10–25%	↑ DI (>25%) → low preload; ↓ DI (<10%) → high filling	Affected by PEEP, compliance
Cardiac contractility	Parasternal long-axis/apical four-chamber	Fractional shortening (FS) 28–45%; eyeball EF ≈ >55%	↓ FS or poor wall thickening → LV dysfunction; hyperdynamic → hypovolemia	Suboptimal images in tachycardia; integrate with clinical signs

CI, collapsibility index; IVC, inferior vena cava; RA, right atrium

Table 4: Lung ultrasound score[45]

Normal			
0	1	2	3
A-pattern	B-pattern	severe B-pattern	Extended consolidations >1 cm
Presence of only A-lines; we accept it as a normal finding	≥3 well-spaced B-lines	crowded and coalescent B-lines ± consolidations limited to the subpleural space (<1 cm)	Pleural effusion present/absent

For a six-area score, the chest surface is divided into three areas in each hemithorax by the anterior axillary line and a line passing through the nipple[46]

1. Upper anterior area (from parasternal to anterior axillary line, above the nipple line).
2. Lower anterior area (from parasternal to anterior axillary line, below the nipple line).
3. Lateral region (from anterior to posterior axillary line).

For each lung area, a 0–3-point score was given (total score: 0–18)

Table 5: Scoring system for pneumonia by lung ultrasound[46]

Score	*Lung USG findings*	*Severity*
1	Pneumonia: Hepatization and shred sign with air bronchogram	Mild
2	Pneumonia with syn-pneumonic effusion	Moderate
3	Pneumonia with effusion, with echogenic particles in the pleural fluid (exudative)	Moderate
4	Empyema	Severe
5	Lung abscess	Severe

USG, ultrasonography

Table 6 shows the lung ultrasound findings in various types of neonatal pneumonia.

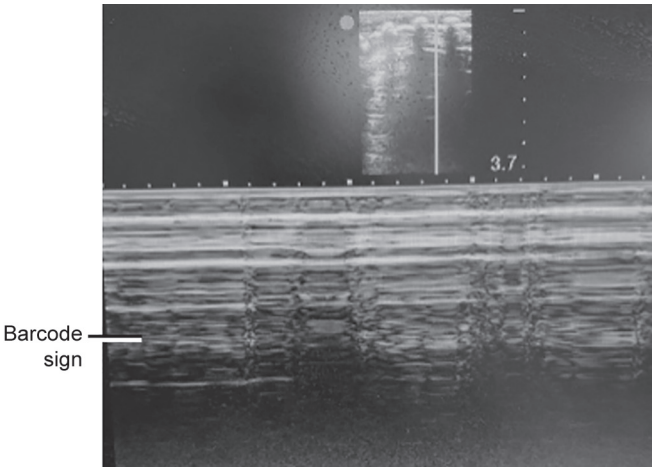


Fig. 4: Lung ultrasound of a neonate in M-mode showing "bar code sign" of pneumothorax. Normal lung in M-mode shows a "Seashore sign" with the top half showing straight horizontal lines (chest wall) and the bottom half with a granular/sandy pattern (sliding lung creates "waves"). Pneumothorax in M-mode may show a barcode or a "Stratosphere sign," where the entire screen shows perfectly straight, parallel horizontal lines from top to bottom

Liver

In patients with umbilical venous catheter (UVC) placement, periodic follow-up ultrasound of the liver region can show migration/misplacement of the lines (Fig. 12).

In neonates with prolonged jaundice and conjugated hyperbilirubinemia, liver sonography could become useful in the latter half of the first month. Bedside liver examination can be useful in infants with clinical features of pulmonary hypertension, congestive cardiac failure, and multi-system deterioration. Some findings in liver sonography can suggest a need for radiological evaluation (Table 7). Figure 5 shows a normal neonatal liver on sonography.[13]

Gastrointestinal Tract

Sonography can provide real-time imaging of the bowel (Fig. 6) and other abdominal viscera with capacity to examine peristalsis, detect ascites, and measure bowel wall thickness/perfusion.[32,47] Some findings could be suggestive of necrotizing enterocolitis (Fig. 7) and should trigger further evaluation (Table 8).

Kidneys, Bladder, Pelvis, Umbilical Region

Sonography can show enlarged kidneys and a radiological evaluation should be done for hydronephrosis, obstruction,

Table 6: Lung ultrasound findings in various types of neonatal pneumonia[46]

Congenital pneumonia	*Community-acquired pneumonia*	*Ventilator-associated pneumonia*	*Atelectasis*
Areas of consolidation, irregular margins	Hepatization with the Shred sign, irregular boundaries of the lesion	Subpleural consolidations	Well-demarcated boundaries of consolidation. Shred sign is less likely
Interstitial syndrome pattern (AIS) with confluent or compact B-lines	Air bronchograms are mostly dynamic	Dynamic air bronchograms	Static air bronchograms and fluid bronchograms in focal atelectasis
Air bronchograms	Pleural line irregularity and absent lung sliding	Lobar consolidation and various degrees of collapse	Pleural line abnormality
Pleural effusion is less common	Syn-pneumonic effusion can be seen	Small areas of para-pneumonic effusion (less common)	A large effusion can be seen. Absent lung sliding in a large area of involvement

Table 7: Normal and altered liver anatomy[13,48]

Category	*Findings on ultrasound*	*Clinical significance*
	Normal liver anatomy	
	Homogeneous, mildly hypoechoic to spleen. Right lobe slightly below the costal margin. The left lobe may cross midline. Portal veins (PVs) show echogenic walls, hepatopetal flow. Hepatic veins show phasic flow to IVC.	Baseline for comparison; ensure proper catheter and vascular orientation.
	Altered liver anatomy	
Hepatomegaly/Congestion	Enlarged liver span, dilated IVC/hepatic veins with pulsatile flow	Congestive heart failure, PPHN, fluid overload
Liver abscess/sepsis	Hypoechoic or complex cystic lesion around a UVC; may contain internal echoes or septa	Bacterial or fungal sepsis, post-UVC infection
Hematoma (UVC or trauma)	Initially hyperechoic → later cystic or mixed lesion; subcapsular or intraparenchymal	UVC malposition or birth injury
PV thrombosis	Echogenic intraluminal focus, absent/reversed Doppler flow	Seen with UVC; check perfusion in other lobe
Ascites	Anechoic fluid around the liver and bowel loops	Sepsis, perforation, cardiac failure
Vascular Doppler patterns	PV: Continuous hepatopetal flow Hepatic vein: Phasic flow with cardiac pulsatility Hepatic artery: Low resistance (RI: 0.6–0.8)	Abnormal: Reversed PV flow (pulmonary hypertension), blunted hepatic vein flow in right heart failure, high RI (ischemia)
	Findings seen in liver disorders	
Cholestasis/Biliary atresia	Absent/small GB, triangular cord sign, echogenic periportal area	Early evaluation in prolonged jaundice
Parenteral nutrition-associated steatosis	Diffusely hyperechoic liver; bright pattern	Common in preterms on long-term TPN
Hepatic calcification	Focal bright echogenic foci with shadowing	TORCH infection, ischemia

UVC, umbilical venous catheter

and vascular flow. Renal views measure kidney size, detect hydronephrosis via urinary tract dilation (UTD) classification (P1–3 for mild–severe; Table 9), and assess renal vein thrombosis in oliguric infants.

These findings can help evaluate the infant in the context of oliguria, urinary tract infections, family history and suspicion of renal vein thrombosis. Table 9 shows UTD classification system, which is currently endorsed by the Society for Fetal Urology, American Urological Association, and American Institute of Ultrasound in Medicine.[49,50] Figure 8 shows USG of a neonate showing urine and sludge in the urinary bladder. It can also help detect bladder size especially in males, bladder volume, and obstruction. Bladder dimensions, urinary volume, and kidney size can be measured.[31,51]

Ultrasound can help rule out an allantoic diverticulum in babies with umbilical discharge. These infants should be evaluated with help from radiologists.

Genitalia

High-frequency linear probe can be used to scan inguinal regions (Fig. 9) for undescended testes (absent in the canal), hydroceles (anechoic fluid), torsion (absent flow), or hernias (bowel loops). Table 10 shows the key sonographic findings in newborn genitalia. These infants should be evaluated with help from radiologists.

Spine

Ultrasound imaging can be very useful for successful performance of LPs; sonography can help locate L5/S1 in transverse and

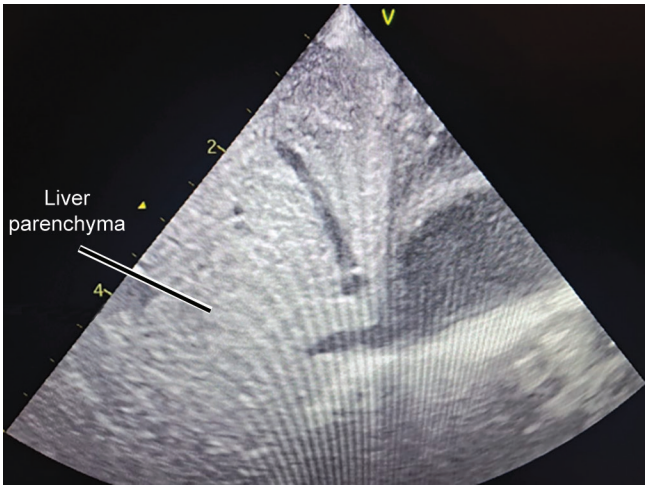


Fig. 5: Normal neonatal liver on sonography showing a homogeneously echogenic parenchyma with a smooth contour

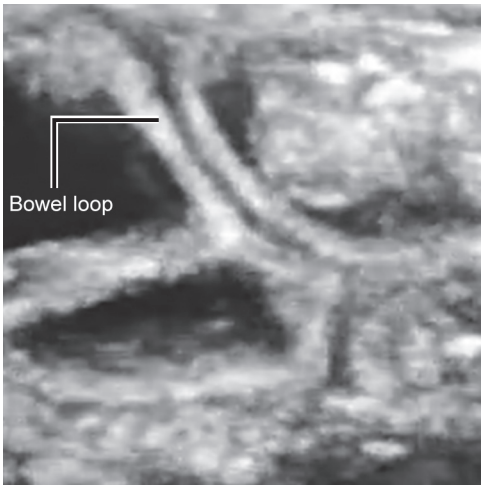


Fig. 6: Gut sonography in an extremely-low-birth-weight neonate demonstrating multiple small bowel loops, consistent with normal gut appearance in a stable preterm infant on day of life 7

longitudinal midline views and determine the appropriate depth of needle insertion. Ultrasonography can also be helpful if there are neurologic signs or lumbosacral skin markers/dermal sinus and should alert the clinicians to consult radiologists to rule out a conus medullaris.[32] Static (pre-marking) vs dynamic (ultrasound guided in real-time) techniques (Table 11) reduce failed LPs by 50–70% in neonates with skin markers. Figure 10 shows transverse (axial) ultrasound view of the neonatal spine.

Developmental Dysplasia of the Hip

Ultrasound allows dynamic and static assessment of hip stability and acetabular development before ossification obscures findings on X-ray. The Graf method (static) and dynamic stress maneuvers are commonly used for evaluation. Alpha angle (>60° normal, <50° abnormal) and β-angle (<55° normal, >77° abnormal) are key parameters in classifying hip morphology (Fig. 11). Table 12 demonstrates the Graf classification for screening DDH in neonates. Universal screening at postnatal 4–6 weeks of age can help detect late dysplasia/disability.

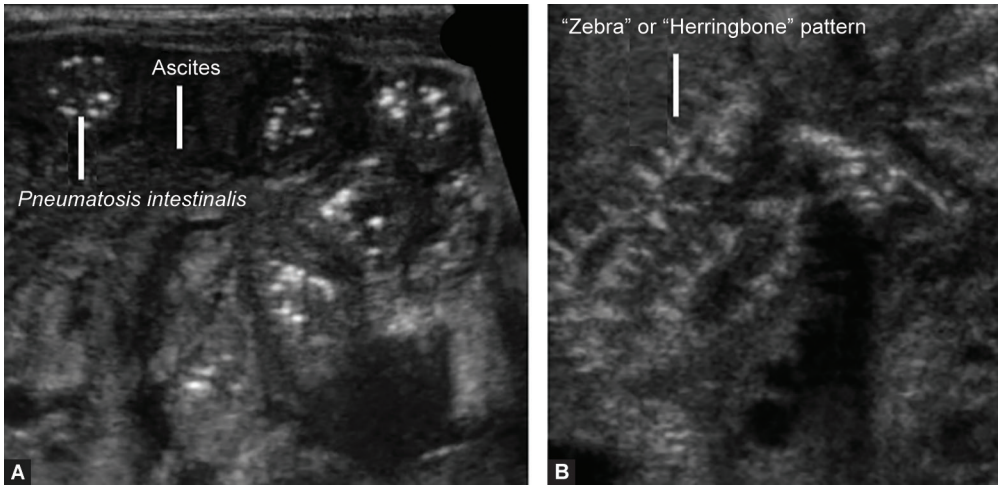
Guided Vascular Access, ETT Position

Sonography can help in correct placement of UVC and percutaneous central catheters (PICCs) lines in neonates; it can help detect catheter tip position.[52] The position of line tip was traditionally determined using radiography, but ultrasound can identify mispositioned catheters more accurately than X-rays in premature infants.[53–55]

Ultrasonography can be used for confirmation of ETT tip positioning in the suprasternal view to ensure it is positioned 1–2 cm above the right pulmonary artery branch.[32,56] Assessment in the longitudinal position over the airway can help determine the distal tip of the ETT, even in premature newborns. Table 13 shows the normal position of ET, umbilical arterial catheter (UAC), UVC, and PICC lines of the upper and lower limbs, as seen in USG and X-rays. Figure 12 shows a UVC and a UAC in an extremely-low-birth-weight infant. A USG film in Figure 13 shows the position of the ETT.

Advantages of Sonography Combined with Traditional Clinical Examination

Bedside sonography is a significant addition to clinical examination. It is time- and cost-effective and serial imaging can be done.



Figs 7A and B: Sonographic diagnosis of NEC. (A) *Pneumatosis intestinalis* is seen as multiple punctate, echogenic foci in the bowel wall. The image also shows some free fluid (ascites) between intestinal loops; (B) Bowel wall edema, seen with increased echogenicity of the small intestinal folds. These findings have been described as forming a "zebra" or "herringbone" pattern

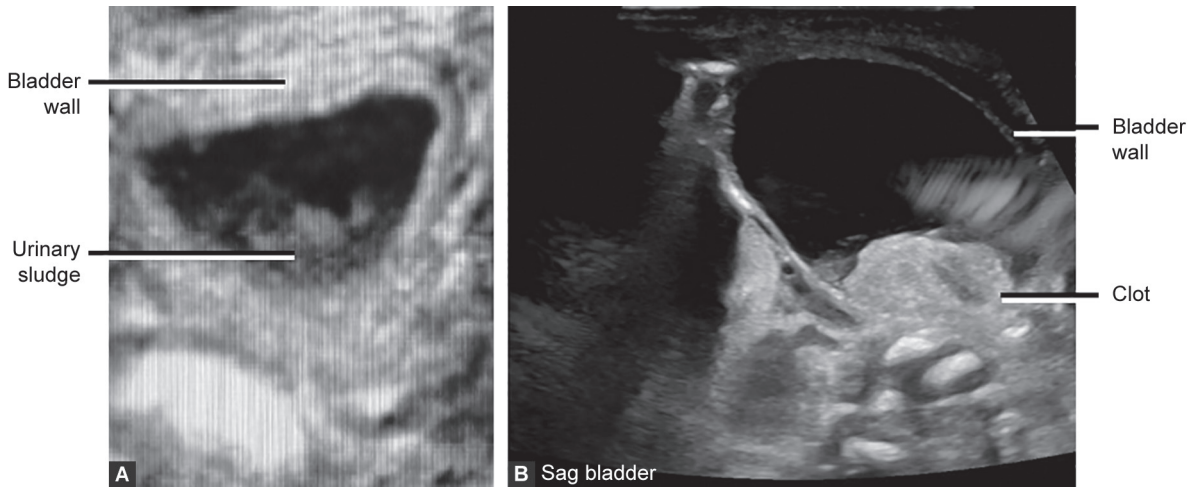
Table 8: Gastrointestinal ultrasound[57]

	Normal gut	*Necrotizing enterocolitis*
Probe tenderness	*Absent*	*Present*
Gut wall echotexture	Gut signature: Five alternating hyperechoic and hypoechoic layers	Loss of gut signature
Gut wall thickness	1–2.6 mm	Increased thickness (>2.6 mm) Later stages show decreased thickness (<1 mm)
Peristalsis	≥10 peristaltic movements/minute	<10 peristaltic movements/minute
Gut wall perfusion	1–9 color signal dots/cm^2	Increased perfusion: >9 color signal dots/cm^2 Specific patterns: Zebra pattern, Y-shaped pattern, ring-shaped pattern. Later stages: Decreased/absent perfusion
Pneumatosis intestinalis	Absent	Present (bright, echogenic dots or lines within the bowel wall)
Portal venous gas	Absent	Present
Fluid collection	Absent (small amounts of simple free fluid can be normal)	Present; complex free fluid is always pathological
Free air collection	Absent	A hyperechoic line seen between the anterior liver and the abdominal wall

Table 9: UTD classification system (Revised 2014)[49,50]

UTD grade	*Findings on postnatal USG*	*Risk level*
UTD P1 (low-risk)	AP diameter 10–15 mm, central calyceal dilation only, normal parenchyma, and ureter	Low
UTD P2 (intermediate-risk)	AP diameter >15 mm, peripheral calyceal dilation, ± ureteral dilation, normal parenchyma	Intermediate
UTD P3 (high-risk)	Same as P2 + abnormal parenchyma (thinned, echogenic) and/or abnormal bladder	High

UTD, urinary tract dilation; USG, ultrasonography



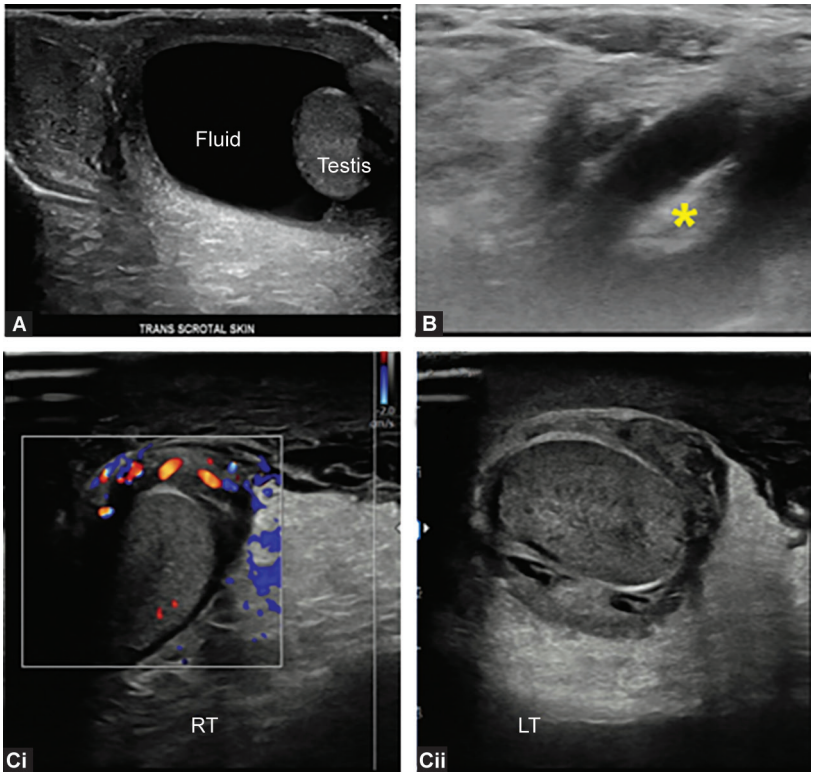
Figs 8A and B: (A) Urinary bladder sonography in a term neonate on postnatal day 12. A low-volume, collapsed bladder is seen with a thickened-appearing wall, smooth contour, containing diffuse, mobile, low-level echogenic debris consistent with urinary sludge; (B) Bladder in a preterm infant with hematuria; a clot was seen in the midline dependent portion of the bladder measuring up to 2.3 cm

There is no need of transporting the baby. Monitoring can be done in real time. There is no ionizing radiation, can be made readily-available, does not require sedation, and is less expensive than magnetic resonance imaging (MRI) and computed tomography.[58–61] Recent models of USG devices are relatively compact and portable, which can be used in essentially all locations where medical care is delivered.

Sonography has been shown to be useful for answering defined urgent clinical questions that require immediate intervention(s) to achieve a desired therapeutic impact.[7] It is dynamic; the same provider performs and interprets the study and can integrate this information within the clinical context over time.[1,7] There are potential concerns, though, arising in (1) lack of training, (2) inadequate collaboration with imaging services, and (3) risk of litigation as the major barriers for its widespread use.[31]

Shortfalls

In 2020, the ECRI committed to address patient-safety challenges and raised concerns regarding clinician-performed sonography.[7] The experts advocated for the needed safeguards for ensuring that the clinicians have the requisite training, experience, and skills. They felt that the lack of sufficient oversight could increase the risk of



Figs 9A to C: Inguinal ultrasounds. (A) Large hydrocele on the left side in a 1-week-old male infant; (B) Inguinal hernia in an 18-day-old female infant. The hernial sac shows fatty tissue but no bowel; (C) Color Doppler ultrasound of the scrotum of a 7-day-old male infant. (Ci) The right testis appears normal in size, echotexture, and vascular perfusion. (Cii) The left testis shows signs of acute torsion, including enlargement, heterogeneous echotexture, and absence of intratesticular blood flow, consistent with compromised perfusion

Table 10: Sonographic assessment of neonatal genitalia[62,63]

Condition	*Key sonographic findings*	*Doppler Flow*
Normal	Homogeneous, mildly hypoechoic testis; epididymis slightly hyperechoic; thin tunica vaginalis	Symmetrical low-velocity flow on both sides
Hydrocele	Anechoic fluid around the testis; may extend into the inguinal canal (communicating)	Normal testicular perfusion
Inguinal hernia	Bowel loops/omentum in scrotum; air–fluid levels; peristalsis	Bowel wall flow preserved (absent = strangulation)
Undescended testis	Testis not palpable in scrotum; hypoechoic ovoid in canal/abdomen	Flow present if viable; absent = atrophic
Testicular torsion	Enlarged, heterogeneous testis; possible "whirlpool" sign	Absent/reduced flow

Normal Doppler indices: RI: 0.6–0.8; symmetrical flow bilaterally. Probe: High-frequency linear (8–15 MHz). Position: Supine with scrotum supported

Table 11: Ultrasound-guided LP in neonates: Static vs dynamic techniques[27,64]

Feature	*Static (Pre-procedure marking)*	*Dynamic (Real-time guidance)*
Probe	Linear or hockey-stick; sterile gel optional	Linear or hockey-stick; sterile cover and gel mandatory
Patient position	Lateral decubitus or sitting; gentle spine flexion	Same; ensure airway patency and stable positioning
Anatomical landmarks	Identify spinous processes, laminae, interspinous gap, posterior dura, and spinal canal	Visualize needle trajectory, posterior dura, and cauda equina movement in real-time
Vertebral level for LP	L3–L4 or L4–L5 interspace (below conus)	Same; confirm with sonography before puncture
Conus medullaris level	Ends at L2–L3 in term; up to L3–L4 in preterm neonates	Identify the conus dynamically in the sagittal plane to avoid injury
Technique	Mark midline, interspace, and measure skin-to-dura depth (5–20 mm, depending on gestation)	Advance the needle under real-time view (in-plane/out-of-plane) until the posterior dura breach is seen
Advantages	Fast, less technical demand; no sterile handling of the probe	Direct needle visualization; highest precision and success rate
Limitations	No live needle tracking; potential for off-axis puncture	Requires expertise, sterile field, and coordination
Best use cases	Routine LPs or limited setup	Difficult LPs (preterm, failed attempts, abnormal anatomy)
Success rate	Higher than landmark-only; moderate	Highest among all methods
Complications	Reduced vs blind LP, but possible misdirection	Minimal; real-time monitoring avoids deep injury

LPs, lumbar punctures

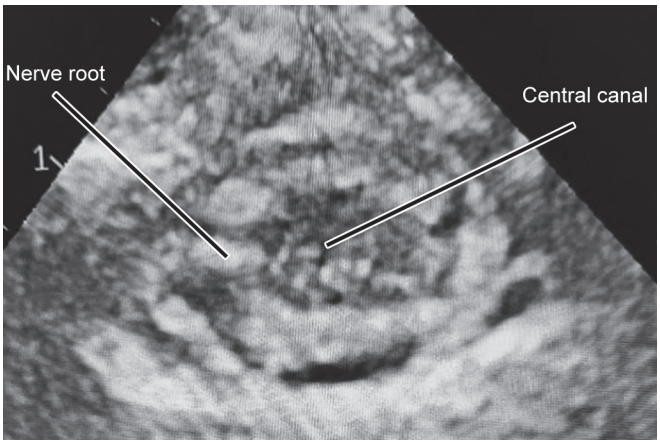
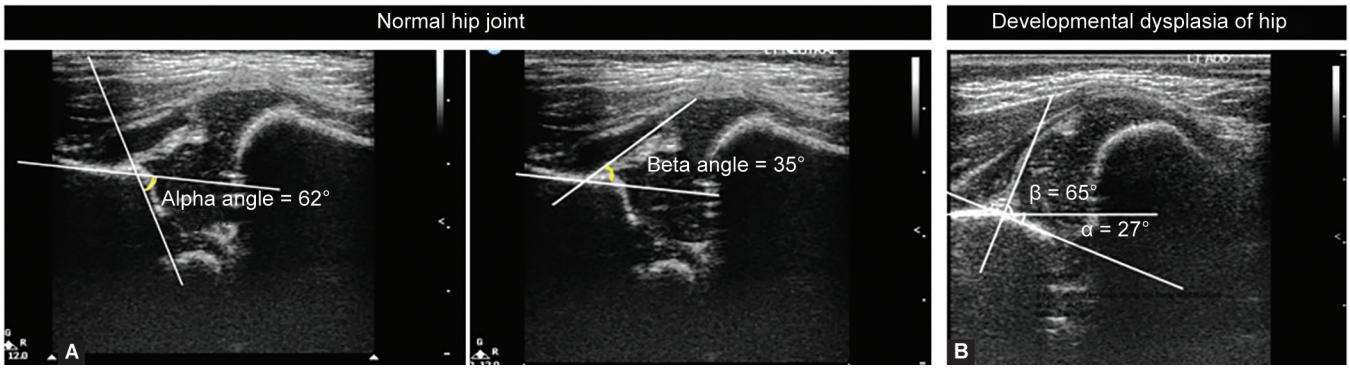


Fig. 10: Transverse (axial) ultrasound view of the neonatal spine (lumbar region) in a 1-week-old term infant, demonstrating normal central canal echo complex and symmetrically positioned bilateral nerve roots



Figs 11A and B: Ultrasound view of the neonatal hip joint. (A) A normal hip; an α-angle measuring >60° and the β-angle of <55°; (B) Dislocated hip

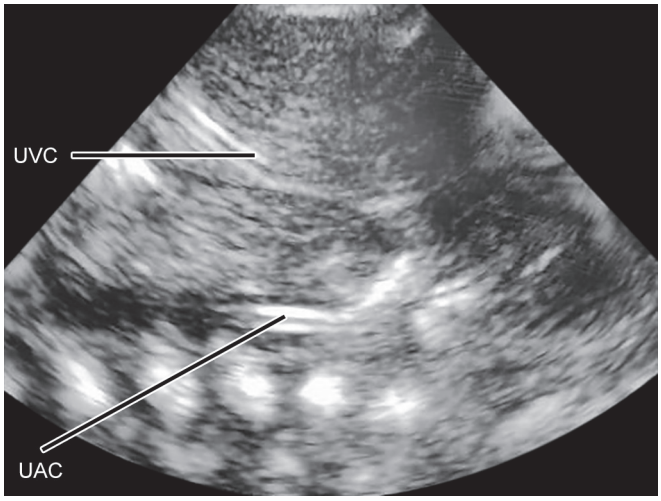


Fig. 12: Sonography showing the position of the UVC and UAC

problems associated with use or lack of use of this technology.[65] To promote safe use of bedside sonography and enhance clinical governance in neonatal and pediatric intensive care units (2020), the ESPNIC Society issued the first international evidence-based guidelines, followed by the AAP clinical and technical reports (2022).[7,28,31]

Other Challenges

In some countries, such as India, legal considerations have prevented the successful implementation of sonographic imaging of fetuses/neonates.[66] The Pre-conception and Pre-natal Diagnostic Techniques (Prohibition of Sex Selection) Act (PC-PNDT), 1994, has helped in curbing female feticide in India, but it has also prevented the use of postnatal sonography, as even the smallest error(s) in fulfilling the legal requirements are viewed seriously by the administrative authorities.[66,67] These laws have also been viewed as impediments in the use of portable ultrasound machines during neonatal transport.[66,68,69]

Future Directions

Sonography has major advantages in its cost-effectiveness (90% for lines/LPs) with a major reduction in NICU mortality by 15–20%. Challenges encompass training barriers, legal hurdles in some countries, and infrastructure needs (dedicated

Table 12: DDH (Graf classification)[70]

Type	*α-angle (°)*	*β-angle (°)*	*Description*
I	≥60	<55	Normal, fully mature hip. The acetabular rim is angular, and the acetabular cup is deep. The cartilaginous roof covers the femoral head.
IIa	50–59	55–77	Physiologically immature at <3 months of age.
IIb	50–59	55–77	Similar to IIa but in infants >3 months. A dysplastic joint requires treatment to prevent further deterioration/dislocation.
IIc	43–49	<77	The hip socket is severely dysplastic and is close to decentering, but the cartilaginous roof still covers the femoral head.
IId	43–49	>77	Resembles IIc, but the hip is decentered. The cartilaginous roof bends cranially.
III	<43	>77	Dislocated femoral head with a shallow acetabulum.
IV	<43	>77	Dislocated femoral head with a severely shallow, dysplastic acetabulum. The cartilaginous roof is markedly displaced.

Table 13: Normal position of intravascular catheters and ETTs

Catheter	*Ideal position of the tip (X-ray)*	*Ideal position of the tip (Ultrasound)*
UAC	High: T6–T9 (descending thoracic aorta, above diaphragm, below left subclavian artery). Low: L3–L5 (abdominal aorta, above bifurcation).	High: Descending thoracic aorta just above the diaphragm.[71] Low: Abdominal aorta above bifurcation.
UVC	T8–T9, at IVC–RA junction, just above the diaphragm. Should not enter RA or remain intrahepatic.	IVC–RA junction, seen via ductus venosus; flow directed centrally toward RA.[72,73]
PICC	Upper limb: T6–T9 (SVC–RA junction). Lower limb: T9–T10 (IVC–RA junction). The tip should be just outside cardiac silhouette.	At the cavo-atrial junction (SVC–RA or IVC–RA), the tip is outside RA.[74]
ETT tip position	Tip position: T1–T2 vertebral level (mid-trachea), Distance from carina: 1–1.5 cm above (≈1 vertebral body).	A distance of 0.5–1 cm between the ET tip and the arch of the aorta suggests its correct placement.[26,75]

ETT, endotracheal tube; PICC, peripherally inserted central catheter; RA, right atrium; UAC, umbilical arterial catheter; UVC, umbilical venous catheter

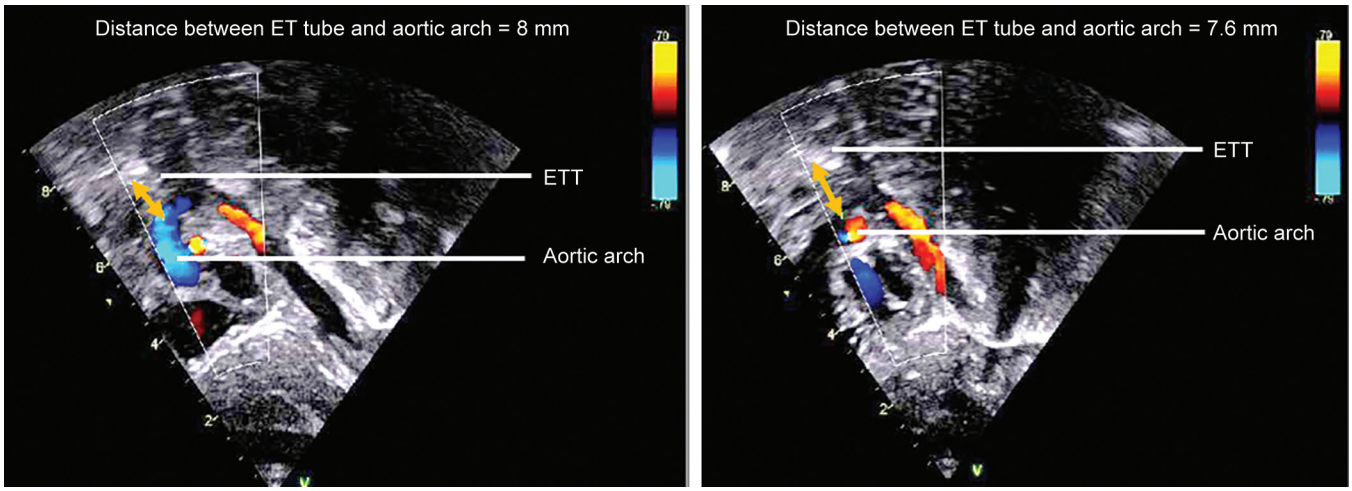


Fig. 13: Sonography (two images from the same patient) shows the hyperlucent endotracheal tube (ET) tip in the trachea. A distance of 5–10 mm (yellow double-ended arrow) between the ET tip and the aortic arch (marked by Doppler signs in this figure) is consistent with optimal mid-tracheal placement

machines with data storage). Future directions involve artificial intelligence-enhanced interpretation and global curricula. Overall, S4 can transform NICU surveillance into a lifesaving, guideline-driven standard.

Key Anatomical Notes

- Conus medullaris: L2–L3 (term), L3–L4 (preterm).
- Safe LP zone: Below conus, typically L3–L4 or L4–L5.
- Typical depth (skin-to-dura): 10–20 mm term; 5–15 mm preterm.

Orcid

Srijan Singh https://orcid.org/0000-0002-2103-5232

References

1. Burdjalov V, Srinivasan P, Baumgart S, et al. Handheld, portable ultrasound in the neonatal intensive care nursery: A new, inexpensive tool for the rapid diagnosis of common neonatal problems. J Perinatol 2002;22(6):478–483. DOI: 10.1038/SJ.JP.7210782.

2. Moore CL, Copel JA. Point-of-care ultrasonography. N Engl J Med 2011;364(8):749–757. DOI: 10.1056/NEJMRA0909487.
3. McLario DJ, Sivitz AB. Point-of-care ultrasound in pediatric clinical care. JAMA Pediatr 2015;169(6):594–600. DOI: 10.1001/JAMAPEDIATRICS.2015.22.
4. Noori S, Seri I. Does targeted neonatal echocardiography affect hemodynamics and cerebral oxygenation in extremely preterm infants? J Perinatol 2014;34(11):847–849. DOI: 10.1038/JP.2014.127.
5. Recker F, Kipfmueller F, Wittek A, et al. Applications of point-of-care-ultrasound in neonatology: A systematic review of the literature. Life (Basel) 2024;14(6):658. DOI: 10.3390/LIFE14060658.
6. Fraga MV, Bhombal S, Juliano C, et al. Neonatal point-of-care ultrasound—Guidelines for training, credentialing and quality assurance. J Perinatol 2025:1–6. DOI: 10.1038/s41372-025-02367-1.
7. Stewart DL, Elsayed Y, Fraga MV, et al. Use of point-of-care ultrasonography in the NICU for diagnostic and procedural purposes. Pediatrics 2022;150(6):e2022060053. DOI: 10.1542/PEDS.2022-060053.
8. Dan AM, Vasilescu DI, Dragomir I, et al. Cranial ultrasonography—Standards in diagnosis of intraventricular hemorrhage and ventricular dilatation in premature neonates. Children 2025;12(6):768. DOI: 10.3390/CHILDREN12060768.
9. Kolnik SE, Sahota A, Wood TR, et al. Cranial point-of-care ultrasound for neonatal providers: A feasibility study. J Ultrasound Med 2024;43(6):1089–1097. DOI: 10.1002/JUM.16437.
10. McNamara PJ, Jain A, El-Khuffash A, et al. Guidelines and recommendations for targeted neonatal echocardiography and cardiac point-of-care ultrasound in the neonatal intensive care unit: An update from the American Society of Echocardiography. J Am Soc Echocardiogr 2024;37(2):171–215. DOI: 10.1016/J.ECHO.2023.11.016.
11. Jagła M, Grudzień A, Tomasik T, et al. Breathe easy, baby, breathe: Lung ultrasound in neonatal critical care. Front Pediatr 2025;13:1631563. DOI: 10.3389/FPED.2025.1631563/BIBTEX.
12. Singh Y, Dauengauer-Kirliene S, Yousef N. Setting the standards: Neonatal lung ultrasound in clinical practice. Diagnostics 2024;14(13):1413. DOI: 10.3390/DIAGNOSTICS14131413.
13. Sohane A, Deshpande S, Nagpal R, et al. Utility of point-of-care ultrasound of the liver in the neonatal intensive care unit: Experience from a case series. Front Pediatr 2025;13:1632908. DOI: 10.3389/FPED.2025.1632908/BIBTEX.
14. Sur A, Gopinathansarasa N. Use of point of care bowel ultrasound (BUS) for diagnosis of suspected necrotizing enterocolitis (NEC): A feasibility study. J Intensive Care Soc 2025;26(4):485–490. DOI: 10.1177/17511437251346376.
15. Clemente EJI, Barber I, Irujo MN, et al. US for evaluation of acute abdominal conditions in neonates. Radiographics 2023;43(2). DOI: 10.1148/RG.220110/SUPPLEMENTAL_8.MP4.
16. Priyadarshi A, Rogerson S, Cruzado R, et al. Neonatologist-performed point-of-care abdominal ultrasound: What have we learned so far? Front Pediatr 2023;11:1173311. DOI: 10.3389/FPED.2023.1173311.
17. Ortenberg J. Urology in the Nursery/NICU New Orleans, Louisiana, USA: LSU Health 2016. Available from: https://laaap.org/wp-content/uploads/2016/08/Urology-in-Nursery-LAAP-1.pdf.
18. Will EP, Fraga MV. Ultrasonography-guided lumbar puncture. Neoreviews 2024;25(8):e527–e529. DOI: 10.1542/NEO.25-8-E527.
19. Gad A, Al-Shouli J, Akomolafe A, et al. Prospective study on ultrasonographic measurement of the spinal canal depth in very low birth weight infants. BMJ Paediatr Open 2025;9(1):e003079. DOI: 10.1136/BMJPO-2024-003079.
20. Kanakamedala AC, Jejurikar NS, Castañeda P. Hip morphology on initial ultrasound predicts hip morphology at one year in developmental dysplasia of the hip. J Child Orthop 2023;17(2):79–85. DOI: 10.1177/18632521221141085.
21. Herrero C, Vidal C, Castaneda P. Acquiring the skills to perform point of care ultrasound of the infant hip through simulation. J Pediatr Orthop Soc North Am 2022;4:467. DOI: 10.55275/JPOSNA-2022-0065.
22. Dasani R, Pai VV, Noh CY, et al. POCUS increases successful placement of peripheral arterial lines in neonates by less experienced providers. Eur J Pediatr 2023;182(11):4977–4982. DOI: 10.1007/S00431-023-05160-4.
23. Koo J. How to use POCUS to place umbilical lines. Neoreviews 2024;25(12):e816–e820. DOI: 10.1542/NEO.25-12-E816.
24. Alonso-Ojembarrena A, Oulego-Erroz I. POCUS for vascular access in neonatology is here to stay. Neonatology 2025;122(5):632–634. DOI: 10.1159/000546404.
25. Sahin O, Tasar S, Colak D, et al. Point-of-care ultrasound for the tip of the endotracheal tube: A Neonatologist Perspective. Am J Perinatol 2024;41(S 01):E2886–E2892. DOI: 10.1055/A-2181-7354.
26. Congedi S, Savio F, Auciello M, et al. Sonographic evaluation of the endotracheal tube position in the neonatal population: A comprehensive review and meta-analysis. Front Pediatr 2022;10:886450. DOI: 10.3389/FPED.2022.886450.
27. Stoller JZ, Fraga MV. Real-time ultrasound-guided lumbar puncture in the neonatal intensive care unit. J Perinatol 2021;41(10):2495–2498. DOI: 10.1038/s41372-021-01152-0.
28. Warburton D, Singh Y, Suryawanshi P, et al. Editorial: POCUS for neonates – Advancing care with point-of-care ultrasound. Front Pediatr 2025;13:1721186. DOI: 10.3389/FPED.2025.1721186.
29. Abrokwa SK, Ruby LC, Heuvelings CC, et al. Task shifting for point of care ultrasound in primary healthcare in low- and middle-income countries – A systematic review. EClinicalMedicine 2022;45:101333. DOI: 10.1016/J.ECLINM.2022.101333.
30. POCUS Cuts Length of Stay by 1.1 days & Reduces costs by 50% | Jan 2025 Report. Accessed November 23, 2025. Available from: https://www.butterflynetwork.com/rutgers-preliminary-findings?srsltid=AfmBOof5YqVRQ-ZBd5-wxDzg1rlruiWuvBERYhOlgWTHIbr_zoEbsD9.
31. Singh Y, Tissot C, Fraga MV, et al. International evidence-based guidelines on point of care ultrasound (POCUS) for critically ill neonates and children issued by the POCUS Working Group of the European Society of Paediatric and Neonatal Intensive Care (ESPNIC). Critical Care 2020;24(1):1–16. DOI: 10.1186/S13054-020-2787-9.
32. Narvey M, Stein N, Elsayed Y. CPS. Published online July 30, 2025. Accessed November 11, 2025. Available from: https://cps.ca/en/documents/position/POCUS.
33. Ecury-Goossen GM, Camfferman FA, Leijser LM, Govaert P, Dudink J. State of the art cranial ultrasound imaging in neonates. J Vis Exp 2015;(96). DOI: 10.3791/52238.
34. Brant JA, Orsborn J, Good R, et al. Evaluating a longitudinal point-of-care-ultrasound (POCUS) curriculum for pediatric residents. BMC Medical Education 2021 21:1 2021;21(1):1–8. DOI: 10.1186/S12909-021-02488-Z.
35. Elsayed Y, Wahab MGA, Mohamed A, et al. Point-of-care ultrasound (POCUS) protocol for systematic assessment of the crashing neonate-expert consensus statement of the International Crashing Neonate Working Group. Eur J Pediatr 2023;182(1):53–66. DOI: 10.1007/S00431-022-04636-Z.
36. Elsayed Y, Sheldon J, Gigolyk S. The impact of respiratory therapist performed point-of-care lung ultrasound on the respiratory care in neonates, Manitoba experience, Canada. Am J Perinatol 2024;41(S 01):E1539–E1545. DOI: 10.1055/S-0043-1768042/ID/JR22OCT1605-15/BIB.
37. Elsayed Y, Narvey M, Lashin A, et al. Point of care lung ultrasound service in neonatal intensive care: Five years of experience in Manitoba, Canada. J Perinatol 2022;42(9):1228–1232. DOI: 10.1038/s41372-022-01455-w.
38. Bhattacharjee I, Volpe MA, Bhattacharya S, et al. Neurodevelopmental impact of early diagnostic imaging in preterm infants: Quantifying risk and the role of point-of-care ultrasound. Front Pediatr 2025;13:1642629. DOI: 10.3389/FPED.2025.1642629/BIBTEX.
39. 2020 Top 10 Health Technology Hazards Executive Brief. Accessed November 14, 2025. Available from: https://home.ecri.org/blogs/ecri-thought-leadership-resources/2020-top-10-health-technology-hazards-executive-brief.

40. ECRI Institute. Adoption of point-of-care ultrasound is outpacing safeguards. Hazard #2—2020 top 10 health technology hazards. Health Devices 2020;1(1):1–7.
41. Chen Q, Xiong W, Jun L. Case report: From diagnosis to therapy: A lung ultrasound-driven precision strategy for neonatal atelectasis management. Front Pediatr 2025;13:1584262. DOI: 10.3389/FPED.2025.1584262/BIBTEX.
42. Chetan C, Majumder S, Debnath A, et al. Neonatal evaluation by extended (12 area) vs. traditional (6 area) lung ultrasound scoring (NEXT-LUS): A prospective observational study. Front Pediatr 2025;13:1638936. DOI: 10.3389/FPED.2025.1638936/BIBTEX.
43. Zaili F, Na H. Lung ultrasound imaging can effectively monitor and guide treatment with pulmonary surfactant for preterm infants of pulmonary consolidation: A prospective observational cohort study. Front Pediatr 2025;13:1634955. DOI: 10.3389/FPED.2025.1634955/BIBTEX.
44. Santos TM, Franci D, Coutinho CMG, et al. A simplified ultrasound-based edema score to assess lung injury and clinical severity in septic patients. Am J Emerg Med 2013;31(12):1656–1660. DOI: 10.1016/j.ajem.2013.08.053.
45. Brat R, Yousef N, Klifa R, et al. Lung ultrasonography score to evaluate oxygenation and surfactant need in neonates treated with continuous positive airway pressure. JAMA Pediatr 2015;169(8):e151797. DOI: 10.1001/JAMAPEDIATRICS.2015.1797.
46. Gupta K, Maheshwari A, Suryawanshi P, et al. Lung ultrasound as a novel tool to assess the severity and management of neonatal pneumonia. Newborn 2024;2(4):291–296. DOI: 10.5005/JP-JOURNALS-11002-0076.
47. De Rose DU, Rallis D, Ibarra-Ríos D, et al. Improving diagnostic interpretability of abdominal ultrasound for neonates with suspected intestinal injury. Front Pediatr 2025;13:1677655. DOI: 10.3389/FPED.2025.1677655.
48. Shkolnik A. Applications of ultrasound in the Neonatal Abdomen. Radiol Clin North Am 1985;23(1):141–156. DOI: 10.1016/S0033-8389(22)02387-9.
49. Nguyen HT, Benson CB, Bromley B, et al. Multidisciplinary consensus on the classification of prenatal and postnatal urinary tract dilation (UTD classification system). J Pediatr Urol 2014;10(6):982–998. DOI: 10.1016/j.jpurol.2014.10.002.
50. Vincent K, Murphy HJ, Twombley KE. Urinary tract dilation in the fetus and neonate. Neoreviews 2022;23(3):e159–e174. DOI: 10.1542/NEO.23-3-E159.
51. Miller LE, Stoller JZ, Fraga MV. Point-of-care ultrasound in the neonatal ICU. Curr Opin Pediatr 2020;32(2):216–227. DOI: 10.1097/MOP.0000000000000863.
52. Barone G, Pittiruti M, Ancora G, et al. Centrally inserted central catheters in preterm neonates with weight below 1500 g by ultrasound-guided access to the brachio-cephalic vein. J Vasc Access 2021;22(3):344–352. DOI: 10.1177/1129729820940174.
53. Fraga MV, Stoller JZ, Glau CL, et al. Seeing is believing: Ultrasound in pediatric procedural performance. Pediatrics 2019;144(5):e20191401. DOI: 10.1542/PEDS.2019-1401.
54. Nguyen J. Ultrasonography for central catheter placement in the neonatal intensive care unit – A review of utility and practicality. Am J Perinatol 2016;33(6):525–530. DOI: 10.1055/S-0035-1569987.
55. Katheria AC, Fleming SE, Kim JH. A randomized controlled trial of ultrasound-guided peripherally inserted central catheters compared with standard radiograph in neonates. J Perinatol 2013;33(10):791–794. DOI: 10.1038/JP.2013.58.
56. Chowdhry R, Dangman B, Pinheiro JMB. The concordance of ultrasound technique versus X-ray to confirm endotracheal tube position in neonates. J Perinatol 2015;35(7):481–484. DOI: 10.1038/JP.2014.240.
57. Maheshwari A, Suryawanshi P, Chetan C, et al. Point-of-care ultrasound to diagnose and monitor the course of necrotizing enterocolitis. Newborn (Clarksville, Md) 2023;2(3):203–213. DOI: 10.5005/JP-JOURNALS-11002-0070.
58. Escourrou G, De Luca D. Lung ultrasound decreased radiation exposure in preterm infants in a neonatal intensive care unit. Acta Paediatr 2016;105(5):e237–e239. DOI: 10.1111/APA.13369.
59. Raimondi F, Yousef N, Migliaro F, et al. Point-of-care lung ultrasound in neonatology: Classification into descriptive and functional applications. Pediatr Res 2021;90(3):524–531. DOI: 10.1038/S41390-018-0114-9.
60. Dietrich CF, Goudie A, Chiorean L, et al. Point of care ultrasound: A WFUMB position paper. Ultrasound Med Biol 2017;43(1):49–58. DOI: 10.1016/j.ultrasmedbio.2016.06.021.
61. Nazarian LN. Sound Judgment. J Ultrasound Med 2012;31(2):187–187. DOI: 10.7863/JUM.2012.31.2.187.
62. Delaney LR, Karmazyn B. Ultrasound of the pediatric scrotum. Semin Ultrasound CT MR 2013;34(3):248–256. DOI: 10.1053/J.SULT.2012.11.010.
63. Luker GD, Siegel MJ, Luker GD, et al. Color Doppler sonography of the scrotum in children. AJR Am J Roentgenol 1994;163(3):649–655. DOI: 10.2214/AJR.163.3.8079863.
64. Trang J, Ku D, Snelling PJ. The utility of point-of-care ultrasound for paediatric lumbar puncture: A narrative review. Emerg Med Australas 2025;37(4):e70103. DOI: 10.1111/1742-6723.70103.
65. ECRI Thought Leadership. Accessed on: November 11, 2025. Available from: https://home.ecri.org/blogs/ecri-thought-leadership-resources.
66. Rath C, Nagpal R, Suryawanshi P. Point-of-care ultrasound in neonatology in India: The way forward. Indian Pediatrics 2023;60(5):351–357. DOI: 10.1007/S13312-023-2879-0.
67. Bhaktwani A. The PC-PNDT act in a nutshell. Indian J Radiol Imaging 2012;22(2). DOI: 10.4103/0971-3026.101114.
68. Carmo KB, Lutz T, Berry A, et al. Feasibility and utility of portable ultrasound during retrieval of sick term and late preterm infants. Acta Paediatr 2016;105(12):e549–e554. DOI: 10.1111/APA.13589.
69. Carmo KB, Lutz T, Greenhalgh M, et al. Feasibility and utility of portable ultrasound during retrieval of sick preterm infants. Acta Paediatr 2017;106(8):1296–1301. DOI: 10.1111/APA.13881.
70. Kang YR, Koo J. Ultrasonography of the pediatric hip and spine. Ultrasonography 2017;36(3):239. DOI: 10.14366/USG.16051.
71. Umbilical artery catheters in the newborn: Effects of position of the catheter tip - Barrington, KJ – 1999 | Cochrane Library. Accessed on: November 12, 2025. Available from: https://www.cochranelibrary.com/cdsr/doi/10.1002/14651858.CD000505/full.
72. D'Andrea V, Prontera G, Rubortone SA, et al. Umbilical venous catheter update: A narrative review including ultrasound and training. Front Pediatr 2022;9:774705. DOI: 10.3389/FPED.2021.774705.
73. Kieran EA, Laffan EE, O'Donnell CPF. Estimating umbilical catheter insertion depth in newborns using weight or body measurement: A randomised trial. Arch Dis Child Fetal Neonatal Ed 2016;101(1):F10–F15. DOI: 10.1136/ARCHDISCHILD-2014-307668.
74. Oleti T, Sankar MJ, Thukral A, et al. Does ultrasound guidance for peripherally inserted central catheter (PICC) insertion reduce the incidence of tip malposition? – A randomized trial. J Perinatol 2019;39(1):95–101. DOI: 10.1038/S41372-018-0249-X.
75. Singh P, Thakur A, Garg P, et al. Normative data of optimally placed endotracheal tube by point-of-care ultrasound in neonates. Indian Pediatr 2019;374–380. PMID: 31102379.

REVIEW ARTICLE

Ethical Challenges in Neonatal Life Support

Srijan Singh[1–3*], Sherri S Buddington[4,5*], Roya Huseynova[2,6,7*]

Received on: 06 October 2025; *Accepted on*: 15 December 2025; *Published on*: 15 January 2026

Abstract

Advancements in neonatal intensive care have dramatically improved survival of extremely preterm and critically ill newborns while simultaneously intensifying ethical dilemmas surrounding futility, parental authority, best interests of the child, and resource allocation. This narrative review examines the application of the four principles of biomedical ethics to contemporary neonatal decision-making, landmark legal cases [Baby Doe, Baby K, Groningen Protocol, Charlie Gard, and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) babies], and persistent controversies in periviability, complex congenital anomalies, neonatal euthanasia, and emerging biotechnologies. Special attention is given to prognostic uncertainty, disability-rights critiques, global disparities, the risks and promises of artificial intelligence (AI) in prognostication, and the ongoing prohibition of heritable genome editing. The article concludes that neonatal ethics remains deeply contested because it confronts fundamental societal values regarding suffering, disability, and the worth of nascent human life, and calls for enhanced international collaboration, evidence-based shared decision-making, and culturally sensitive consensus guidelines, with a potential leadership role for the Global Newborn Society (GNS).

Keywords: Artificial intelligence, Baby Doe, Baby K, Best interests, Charlie Gard, CRISPR, Disability rights, Extreme prematurity, Futility, Genome editing, Groningen protocol, Infant, Neonatal ethics, Neonatal euthanasia, Neonate, Newborn, Parental autonomy, Periviability, Prognostic uncertainty, Quality of life, Shared decision-making, Withholding/withdrawing treatment.

Newborn (2025): 10.5005/jp-journals-11002-0144

Key Points

- Survival of periviable (22–25 weeks) and critically ill newborns has increased, but decisions about initiation or continuation of life-sustaining treatment are increasingly ethically complex due to high risks of severe neurodevelopmental impairment and prognostic uncertainty.
- Landmark legal cases and differing national frameworks [the Baby Doe rules, The Emergency Medical Treatment and Labor Act (EMTALA) obligations in the USA, and the Netherlands Groningen Protocol] reveal irreconcilable tensions between parental autonomy, medical judgment, futility concepts, and state authority in end-of-life decisions.
- Quality-of-life assessments and predictions of "intolerable" disability continue to provoke disability-rights critiques and accusations of implicit discrimination, highlighting the subjective and value-laden nature of "best interests" determinations.
- Emerging technologies—artificial intelligence (AI) for individualized prognostication and CRISPR-based gene therapies—offer clinical promise but introduce new ethical risks, including algorithmic bias, erosion of human judgment, and the danger of reviving eugenic practices through heritable genome editing.
- Greater international, multidisciplinary, and culturally sensitive collaboration is urgently needed to reduce moral distress, standardize ethical reasoning, improve transparency, and ensure equitable access to both intensive and palliative neonatal care worldwide.

[1]Department of Neonatology, Yashoda Medicity, Indirapuram, Uttar Pradesh, India

[2]Global Newborn Society, Harrison, New York, United States of America

[3]GNS Forum for Transgenerational Inheritance, New York, United States of America

[4]Government Relations and Healthcare Policy Group, Shreveport, Los Angeles, United States of America

[5]Capitol Strategy Group, Shreveport, Los Angeles, United States of America

[6]Member, World Medical Law Society

[7]Neonatologist, King Saud Medical City Riyadh, Kingdom of Saudi Arabia

Corresponding Authors: Srijan Singh, Department of Neonatology, Yashoda Medicity, Indirapuram, Uttar Pradesh, India; Global Newborn Society, Harrison, New York, United States of America; GNS Forum for Transgenerational Inheritance, New York, United States of America, Phone: +91 9811209371, e-mail: srijanstar89@gmail.com; Sherri S Buddington, Government Relations and Healthcare Policy Group, Shreveport, Louisiana, United States of America; Capitol Strategy Group, Shreveport, Louisiana, United States of America, e-mail: sherri@sherribuffington.com; Roya Huseynova, Global Newborn Society, Harrison, New York, United States of America; Member, World Medical Law Society; Department of Neonatology, King Saud Medical City Riyadh, Kingdom of Saudi Arabia, e-mail: huseynova_roya@yahoo.com

How to cite this article: Singh S, Buddington SS, Huseynova R. Ethical Challenges in Neonatal Life Support. Newborn 2025;4(4):194–202.

Source of support: Nil

Conflict of interest: None

Ethical Challenges in Neonatal Life Support

Introduction

The survival of extremely preterm and critically ill newborns has increased dramatically over the past four decades but so has the frequency of ethically fraught decisions. Clinicians, families, ethicists, and policymakers must repeatedly confront questions such as: When is continued treatment futile or disproportionately burdensome? Who has the ultimate authority to decide—parents, physicians, courts, or the state? How should scarce resources and societal values

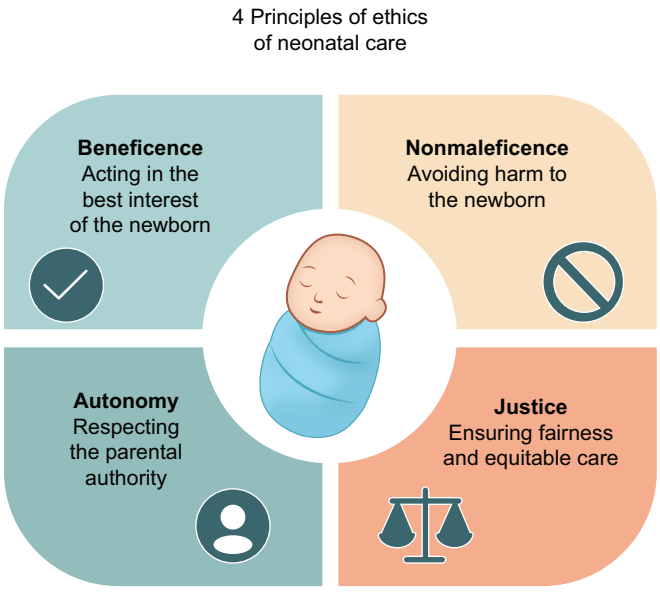


Fig. 1: Principles of ethical neonatal care

influence individual cases? These dilemmas are not new, but their scope and visibility have grown with technological capability.

Medical ethics has been traditionally centered on three key levels:

1. Metaethics: Origin and meaning of ethical principles;
2. Normative ethics: Agreement about standards that regulate in-/appropriate conduct;
3. Applied ethics: Application to specific controversies such as abortion.

In neonatal care, the ethical analysis is typically extended to cover Beauchamp and Childress's four central principles (Fig. 1):[1]

1. Respect for Autonomy

In neonates, autonomy is necessarily proxy-based. Parents are generally presumed to be the appropriate surrogate decision-makers, but their authority is not unlimited and may be overridden when choices clearly contravene the child's best interests.

2. Beneficence

Clinicians are obligated to act in ways that promote the newborn's welfare. Determining benefit, however, becomes contested when survival is accompanied by profound neurological impairment.

3. Non-maleficence ("Do No Harm")

Continued aggressive treatment in cases of predicted intolerable suffering or irreversible total dependence may violate this principle. The distinction between "killing" and "allowing to die" remains philosophically and emotionally charged.

4. Justice

Distributive justice raises questions about the allocation of neonatal intensive care unit (NICU) beds, expensive technologies, and specialist time, especially when outcomes are poor and costs are high.

In this aerial view, three major difficulties have been noted:

1. Moral dilemmas: A care provider feels uncertain when more than one way may feel appropriate to proceed; each of these might be weighted on one of the four central principles (above);
2. Moral uncertainties: The presenting diagnosis is unclear, with differing outcomes;
3. Moral distress: The viability at a particular gestational age or with a particular diagnosis is uncertain, and a care provider feels uncertain about providing all possible care.

Landmark Cases and Legal Frameworks

Baby Doe (USA, 1982)

An infant with Down syndrome and esophageal atresia was denied surgery at parental request. After intense public and political reaction, the Reagan administration issued the "Baby Doe regulations" under the Child Abuse Amendments of 1984, mandating treatment unless the infant is irreversibly comatose, treatment is futile, or treatment would merely prolong dying.[2,3]

Baby K (USA, 1992–1994)

An anencephalic infant was kept on mechanical ventilation for over 2 years because the mother insisted on life-sustaining treatment.[4–8] The case highlighted tensions between parental rights and medical recommendations of futility and has helped shape interpretations of the Emergency Medical Treatment and Active Labor Act (EMTALA). Courts have ruled that EMTALA requires hospitals to provide ventilatory support in emergency settings even when physicians consider it non-beneficial.

Terri Schiavo (USA, 1990–2005)

Although not a neonate, the prolonged legal battle over the withdrawal of artificial nutrition in a woman in a persistent vegetative state influenced public and legislative understanding of "right to life" vs "right to die" issues and reinforced the importance of advance directives.[9–11]

Emtala (USA, 1986)

This US federal law mandates stabilizing treatment for any patient presenting to an emergency department. In neonatal cases (e.g., Baby K), it has been interpreted to require continued ventilatory support for anencephalic infants if respiratory distress constitutes an "emergency medical condition," regardless of long-term prognosis.[12–17]

Groningen Protocol (Netherlands, 2004–Present)

Developed at Groningen University Medical Center and later formalized nationally, this protocol permits active euthanasia of newborns under strict conditions: Hopeless prognosis, unbearable suffering, parental consent, independent review, and transparent reporting.[18–20] Although rarely used (fewer than five cases annually), its existence marks a radical departure from the Anglo-American tradition of withholding/withdrawing only, never intentional ending of life.

Figure 2 shows a timeline of the above landmark events. Figure 3 shows the complex ethical landscape of neonatal medicine, where decisions involving life-sustaining treatment, resource allocation, parental involvement, and best-interest determinations are made by a multidisciplinary team of physicians, nurses, and allied health professionals.

Specific Ethical Dilemmas in Neonatal Subpopulations

Periviability (22–25 Weeks Gestational Age)

The borderland of viability has shifted downward with each decade, creating an ethical "gray zone" where survival is possible but severe

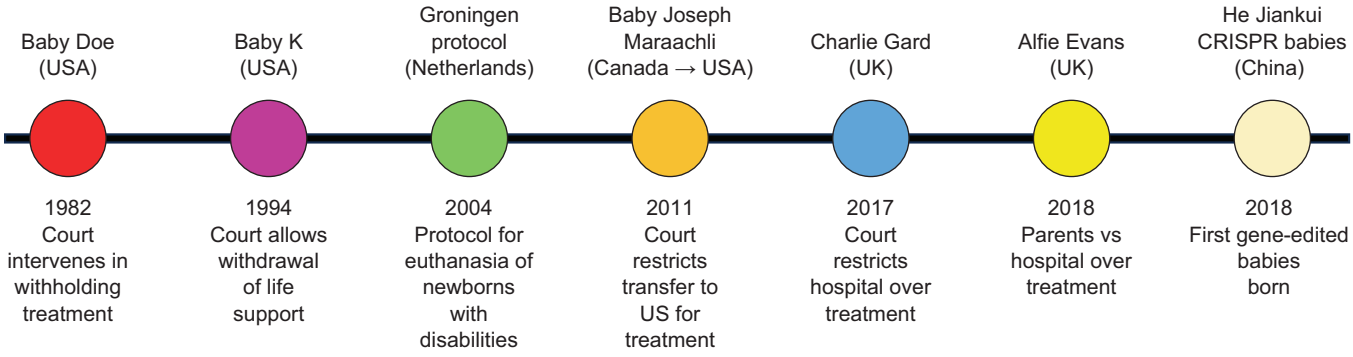
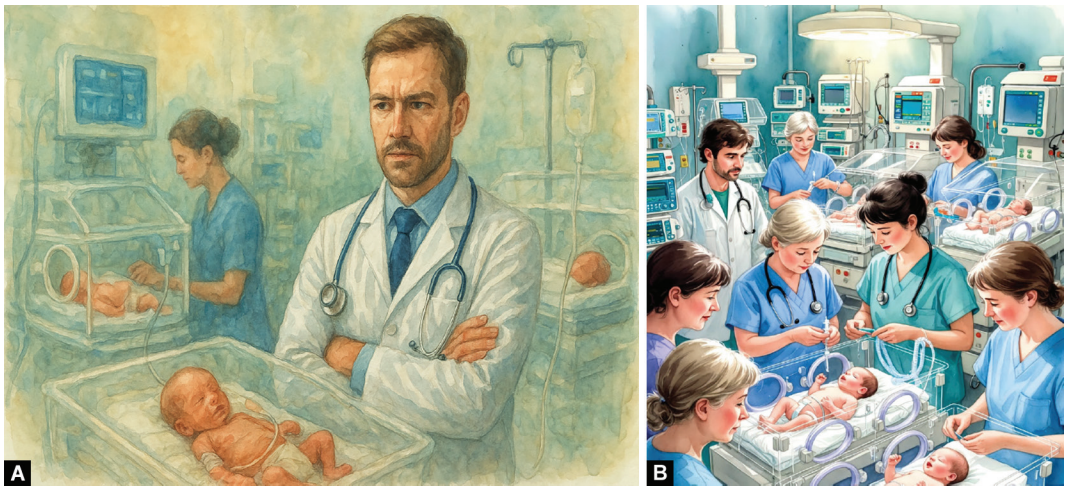


Fig. 2: Landmark events that have helped in our understanding of ethical issues in neonatal care



Figs 3A and B: A multidisciplinary NICU team providing specialized medical and nursing care to preterm and critically ill newborns in a modern tertiary-level facility. The images above illustrate the complex ethical landscape of neonatal medicine, where decisions involving life-sustaining treatment, resource allocation, parental involvement, and best-interest determinations are made collaboratively by physicians, nurses, and allied health professionals. Considering that these illustrations seek to convey human emotions and not quantifiable data, we have used an AI program to design these figures

Table 1: Survival and intact survival rates in periviability (2020–2025 data)

Gestational age	*Active resuscitation survival to discharge*	*Survival without severe impairment (intact survival)*	*Major source*
22 weeks	28–55%	4–16%	NICHD NRN 2022, VON 2024, EPIPAGE-3[21,22]
23 weeks	55–75%	20–38%	NICHD NRN 2022, EXPRESS-2, Japanese Neonatal Network[21–23]
24 weeks	75–88%	45–65%	NICHD NRN, VON, Swiss Neonatal Network[23]
25 weeks	88–94%	65–80%	All major networks

neurodevelopmental impairment is common. At 22 weeks, intact survival is <10% in most registries; at 23 weeks, 20–35%; at 24 weeks, >50%.[24–27] Families and clinicians must decide whether to initiate resuscitation in the delivery room or provide comfort-focused care only. Guidelines vary widely: Some countries (e.g., Japan, parts of the US) advocate active treatment from 22^{+0} weeks; others (e.g., Netherlands, France, Switzerland) recommend parental choice with non-resuscitation as a legitimate option below 24–25 weeks. Key ethical tensions include the moral weight of small survival chances vs the risk of prolonged suffering and lifelong disability, the reliability of early prognostic markers, and the potential for implicit bias when counseling families from disadvantaged backgrounds.[28] Table 1 shows the survival and intact survival rates in periviability.

Figure 4 shows an algorithm for ethical decision-making for periviable births (23^{+0}–24^{+6} weeks).

Complex Multiple Congenital Anomalies

Newborns with conditions such as trisomy 13 or 18, severe hypoxic-ischemic encephalopathy with multicystic encephalomalacia, hypoplastic left heart syndrome combined with genetic syndromes, or extensive neural tube defects often present overlapping issues of uncertain prognosis and cumulative burden. Unlike isolated anomalies amenable to corrective surgery, these cases typically involve irreversible limitations in cognition, communication, and independence. Ethical discourse has shifted from older "lethal anomaly" terminology to recognition that many children survive

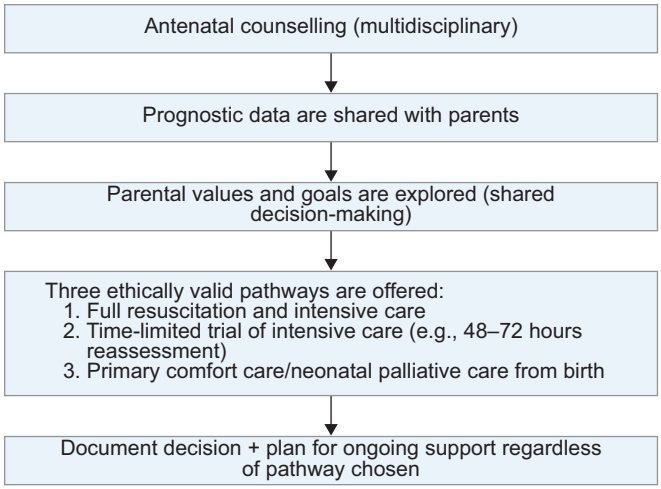


Fig. 4: Ethical decision-making algorithm for periviable birth (23^{+0}–24^{+6} weeks)

years or decades, albeit with profound dependency. Decision-making must balance parental hopes, the child's expected subjective experience, resource implications, and avoidance of discriminatory assumptions that certain lives are "not worth living."[29]

Advances in prenatal screening and diagnostic technologies (cell-free DNA testing, detailed anatomy ultrasound, fetal MRI, and whole-exome sequencing) now permit detection of major congenital anomalies, chromosomal disorders, or genetic syndromes as early as the first trimester. While early detection offers parents informed reproductive choices—including continuation of pregnancy with preparation, fetal therapy, or, in some jurisdictions, termination—these findings also impose profound ethical challenges in neonatal care. Families frequently experience anticipatory grief, guilt, stigmatization, or pressure from clinicians or society toward one particular decision, whereas others feel abandoned if offered only palliative-care pathways rather than aggressive postnatal intervention. Neonatal teams have an ethical duty to provide non-directive, compassionate, and continuity-based counseling that begins prenatally, respects diverse moral views on disability and reproductive autonomy, avoids prognostic overconfidence, and ensures that any birth plan (curative, comfort-focused, or perinatal hospice) is developed collaboratively with parents and consistently honored after delivery, thereby protecting parental authority while safeguarding the future child's dignity and best interests.

Neonatal Pain and the Ethics of Palliative/Comfort Care

Infants, even the most premature, experience pain and stress mediated through mature nociceptive pathways. Repeated procedural pain in the NICU is associated with adverse long-term neurodevelopmental and behavioral outcomes. Withholding analgesia or sedation during end-of-life care on the grounds that the infant is "pre-viable" or "dying anyway" is ethically indefensible. Concurrently, the principle of double effect permits administration of opioids or sedatives in doses required for comfort even if respiratory depression and hastened death are foreseeable (though not intended) side effects. High-quality neonatal palliative care integrates pain neuroscience, family-centered communication, and interdisciplinary expertise; it is not "giving up" but a positive ethical obligation when continued curative intent would violate non-maleficence.[30–38]

Persistent Complexities (Fig. 5)

- Defining "futility" remains subjective and value-laden.
- Quality-of-life judgments risk discrimination against persons with disabilities.
- Religious and cultural diversity among families and staff complicates consensus.
- Prognostic uncertainty in extreme prematurity (22–25 weeks of gestation) makes early decisions particularly difficult.
- Disability-rights critique: Many advocates argue that decisions based on predicted "poor quality of life" devalue disabled lives and echo eugenic thinking.

Religious Sensitivities

In neonatal care, religious sensitivities frequently influence critical decisions about life-sustaining treatment, such as Jehovah's Witness parents refusing blood products for a preterm infant with severe anemia or Orthodox Jewish families requesting accommodation of Sabbath restrictions during resuscitation. Ethically, clinicians must respect parental religious autonomy while upholding the infant's best interests; this tension is typically addressed through early proactive dialog, hospital faith-liaison support, ethics committee consultation, and, in rare irreconcilable cases, temporary court-ordered treatment to prevent imminent harm.

Social Inclusion of Racial and Indigenous/Native Communities

Racialized, Black, Indigenous, and Traveler families often experience systemic barriers in neonatal care, including implicit bias, language discordance, historical mistrust, and geographic distance from tertiary centers, resulting in lower rates of antenatal steroids, kangaroo care uptake, and follow-up developmental services. Ethical neonatal practice demands cultural safety training, community co-design of services, routine use of professional interpreters, and targeted outreach programs to reduce disparities and affirm equitable moral regard for every newborn.

Families with Same-gender Parents

Same-gender parent families in the NICU may encounter heteronormative assumptions in medical forms, visitation rules, or staff communication ("which one is the real mother?"), which can intensify emotional distress during an already vulnerable period. Ethical care requires gender-neutral language, inclusive parent designation policies, staff sensitivity training, and immediate correction of any discriminatory practice to ensure both parents feel fully recognized and supported in bonding and decision-making for their infant.

Infants Born to Parents Facing Dire Financial Challenges

Extreme poverty profoundly affects neonatal outcomes through late prenatal care, maternal malnutrition, housing instability, and inability to attend follow-up appointments; ethically, the neonatal team must avoid judgmental attitudes and instead adopt a social-determinants-of-health framework, connecting families to financial aid, transportation vouchers, and community resources while advocating for policies that decouple access to intensive care from socioeconomic status.

Fig. 5: Conundrums in neonatal ethics

Infants Born to Undocumented Immigrant Families

Undocumented parents often delay or avoid seeking neonatal care because of deportation fears, leading to preventable morbidity; neonatal ethics committees widely agree that the infant's right to emergency and ongoing treatment supersedes immigration enforcement concerns. Units should implement firewall policies separating clinical care from immigration reporting, provide confidential social-work support, and partner with legal-aid organizations to protect family unity and the child's welfare.

Infants Born to Parents with Substance Dependence

Neonates exposed to opioids or other substances risk neonatal abstinence syndrome and child-protection involvement; ethical care rejects stigmatizing labels ("addict baby") and instead embraces non-punitive, trauma-informed approaches that encourage maternal presence, rooming-in, breastfeeding when safe, and family-centered addiction treatment to promote bonding and reduce long-term removal from parental custody whenever it is consistent with infant safety.

The Emerging Role of AI in Neonatal Ethics

Artificial intelligence is beginning to influence neonatal decision-making in ways that simultaneously promise benefit and raise profound ethical concerns. Machine-learning algorithms can now integrate genomic, physiologic, imaging, and sociodemographic data to generate highly individualized prognostic predictions for extreme prematurity, hypoxic-ischemic encephalopathy, or complex congenital conditions—often outperforming traditional scoring systems. Such tools could reduce subjective bias in counseling, promote transparency, and support parents facing time-pressured decisions. However, risks are substantial: (1) opaque "black-box" models may erode trust and accountability; (2) training datasets drawn predominantly from high-income countries can perpetuate or amplify inequities when deployed in low-middle-income countries; (3) over-reliance on probabilistic outputs threatens to supplant human judgment and parental values with algorithmic authority; (4) predictive scores may subtly shift clinician language from shared deliberation to deterministic pronouncements ("the AI gives this baby only a 9% chance of intact survival"), potentially coercing families; and (5) commercial interests behind many AI tools create conflicts of interest. Ethically robust integration of AI, therefore, demands transparent validation across diverse populations, explicit disclosure of limitations, preservation of clinician and parental primacy in value-laden choices, and global governance frameworks to prevent exacerbation of existing disparities. Rather than replacing moral reasoning, AI should serve as a humble servant of compassionate, context-sensitive decision-making. The Global Newborn Society (GNS) and similar bodies have an urgent role in developing international standards for the responsible design, validation, and clinical deployment of AI in neonatal ethics.

Gene Editing in Neonatal Ethics

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based technologies have transformed the treatment of single-gene disorders (e.g., spinal muscular atrophy, sickle-cell disease) through approved somatic gene therapies delivered in the neonatal period. These are ethically comparable to other high-cost, high-benefit interventions, with justice and access as the primary concerns.

In contrast, heritable (germline) editing remains prohibited by major international consensus statements [World Health Organization (WHO) 2021, US National Academies 2017, Nuffield Council 2018] due to off-target risks, irreversible intergenerational effects, and the danger of sliding from therapy to enhancement, thereby reviving eugenic practices and deepening global inequity.[39–41]

Prenatal or neonatal germline intervention currently lacks sufficient safety, efficacy, and societal legitimacy to be ethically permissible. Clinicians must provide accurate information, resist pressure for offshore or experimental use, and uphold the moratorium until rigorous, equitable, and transparent global governance is established. The GNS should continue to affirm that heritable genome editing in newborns is presently morally unacceptable.

Ethics of Prenatal Gene Editing

Somatic prenatal gene editing (non-heritable) is increasingly accepted for severe, early-onset disorders (e.g., spinal muscular atrophy, thalassemia) when postnatal treatment is too late, risks are low, and maternal informed consent is genuine. Concerns are maternal risk, long-term uncertainty, and prohibitive cost.

Germline prenatal gene editing (heritable changes) is currently ethically unacceptable and prohibited by global consensus (WHO 2021, International Society for Stem Cell Research 2021, National Academies/Royal Society). Core objections are as follows:

- Non-consensual intergenerational effects,
- Unknown off-target risks,
- Slippery slope to enhancement and eugenics,
- Exacerbation of global inequity.

Clinicians must clearly state that heritable prenatal editing remains morally impermissible.

CRISPR Babies Case (2018)

Chinese scientist He Jiankui announced in November 2018 that he had created the world's first gene-edited babies: twin girls (Lulu and Nana) and later a third child. Using CRISPR-Cas9 on *in vitro* fertilization embryos, he disabled the CC-receptor 5 (*CCR5*) gene, aiming to confer resistance to the human immunodeficiency virus (HIV; father was HIV-positive).[42–44]

Key issues were as follows:

- Germline (heritable) editing
- Medically unnecessary (standard IVF prevents HIV transmission)
- Inadequate consent, no proper ethics approval, secret experiment
- Off-target mutations and mosaicism risks are unknown.

The 2018, the CRISPR babies case was condemned all over the world as reckless and unethical. He Jiankui was sentenced to 3 years in prison (2019), fired, and banned from reproductive research and China tightened regulations.[44] The case remains the only confirmed instance of deliberate heritable human genome editing and is widely cited as a major ethical breach.[45–48]

Controversies in Neonatal Ethics

- Active euthanasia: Netherlands' Groningen Protocol (~15 cases since 2005) permits lethal injection for unbearable suffering (e.g., severe epidermolysis bullosa). Elsewhere, identical cases (e.g., Baby Isaiah, Texas 2013) result in prolonged dying or criminal charges.
- Disability-rights critique: Parents of baby Joseph Maraachli (Canada/United States 2011) fought ventilator withdrawal; after transfer to St. Louis, he lived eight more months at home. This is cited by advocates as proof that "poor quality of life" predictions are often biased.
- Trisomy 13/18 treatment: Baby Miraculous (Italy 2019) and Baby Lillian (US 2021) survived years with full trisomy 13 after parents demanded surgery against medical advice that the condition was "incompatible with life."
- Cost/rationing: In the case of Charlie Gard (UK 2017) and Alfie Evans (UK 2018), the NHS refused further experimental treatment, which was upheld by courts partly on resource grounds.[49–54]
- Research ethics: SUPPORT oxygen trial (US 2010–2013) randomized extremely preterm infants to high- vs low-oxygen ranges without disclosing increased risk of death or blindness in either arm. It led to federal consent reforms.[55,56]

Table 2: Key legal and policy frameworks in neonatal end-of-life decisions

Jurisdiction	*Withholding/withdrawing allowed*	*Active euthanasia allowed*	*Landmark case/Law*
United States	Yes	No	Baby Doe Rules (1984), EMTALA, Baby K[3,6,12,16,17]
United Kingdom	Yes	No	Charlie Gard (2017), Alfie Evans (2018)[54]
Netherlands	Yes	Yes (Groningen Protocol)	Groningen Protocol (2005–present)[18–20]
Belgium	Yes	Yes (2002 law extended to minors in 2014)	–
Canada	Yes	No	Baby Joseph Maraachli (2011)[57,58]

These high-profile cases expose irreconcilable clashes over sanctity vs quality of life, parental authority vs medical judgment, and overt vs covert rationing. Table 2 shows the key legal and policy frameworks in neonatal end-of-life decisions.

Current Challenges

1. Rising survival of periviable infants (22–23 weeks) with high rates of severe morbidity.
2. Disparities in access to high-level NICUs by geography and socioeconomic status.
3. Moral distress and burnout among neonatal staff repeatedly involved in end-of-life decisions.
4. Inconsistent international guidelines (e.g., active euthanasia permissible in the Netherlands and Belgium; strictly prohibited elsewhere).
5. The commodification risk of emerging technologies (e.g., artificial placentas, *ex utero* gestation systems).

Future Directions

- Development of more granular, evidence-based outcome data to reduce prognostic uncertainty.
- Structured shared decision-making models incorporating decision aids and ethics consultation.
- International consensus efforts (e.g., through the International Society for Neonatal Intensive Care Ethics).
- Integration of palliative care from the moment of birth in high-risk cases.
- Exploration of conditional or time-limited trials of intensive care with predefined reassessment points.

The Potential Role of the GNS

The GNS, with its explicit mission to improve newborn health worldwide while respecting cultural diversity, is uniquely positioned to foster ethical dialogue across borders. Global Newborn Society could: (1) develop and regularly update evidence-based, culturally sensitive consensus statements on controversial practices; (2) create an international registry of difficult cases (anonymized) to improve transparency and learning; (3) offer online training modules on ethical reasoning for low- and middle-income settings where resources are severely constrained; (4) advocate for equitable access to both high-technology care and high-quality palliative/perinatal hospice services; and (5) serve as a neutral platform for dialog between disability-rights organizations, religious communities, and neonatologists. By emphasizing global rather than purely Western frameworks, GNS could help prevent the export of one region's moral absolutes while promoting minimum standards of respect for newborn life and family dignity.

Conclusion

Neonatal ethics will remain contested terrain because it forces society to confront its deepest values about disability, suffering, parenthood, and the meaning of a life worth living. While technological capacity continues to advance, wisdom requires ongoing, humble, multidisciplinary conversation that honors both the fragility and the dignity of the newest members of the human family.

References

1. Beauchamp TL, Childress JF. Principles of Biomedical Ethics. Oxford University Press; 2019;8:512. Accessed on: December 7, 2025. Available from: https://global.oup.com/ushe/product/principles-of-biomedical-ethics-9780190640873?cc=co&lang=en.
2. Placencia FX, McCullough LB. The history of ethical decision making in neonatal intensive care. J Intensive Care Med 2011;26(6):368–384. DOI: 10.1177/0885066610393315.
3. The Baby Doe Rules (1984). Embryo Project Encyclopedia. Accessed on: December 7, 2025. Available from: https://embryo.asu.edu/pages/baby-doe-rules-1984.
4. Legal case review: Case summary: In the matter of Baby "K" (4th Cir. 1994). Self-Insurance Programs. Accessed on: December 7, 2025. Available from: https://flbog.sip.ufl.edu/risk-rx-article/legal-case-review-case-summary-in-the-matter-of-baby-k-4th-cir-1994/.
5. Brown K. In the matter of Baby K: The Fourth Circuit stretches EMTAIA even further. Mercer Law Rev 1996;47(4). Accessed on: December 7, 2025. Available from: https://digitalcommons.law.mercer.edu/jour_mlr/vol47/iss4/9.
6. Furrow BR. Treatment of an anencephalic infant: The case of Baby K. Int J Risk Saf Med 1995;7(1):65–68. DOI: 10.3233/JRS-1995-7107.
7. U.S. District Court, E.D. Virginia, Alexandria Division. In re Baby K. Fed Suppl 1993;832:1022–1031. PMID: 11648613.
8. In the matter of Baby "K" (three cases), 16 F.3d 590 (4th Cir. 1994). Justia. Accessed on: December 7, 2025. Available from: https://law.justia.com/cases/federal/appellate-courts/F3/16/590/492033/.
9. Mueller PS. The Terri Schiavo saga: Ethical and legal aspects and implications for clinicians. Pol Arch Med Wewn 2009;119(9):574–581. DOI: 10.20452/PAMW.771.
10. Hook CC, Mueller PS. The Terri Schiavo saga: The making of a tragedy and lessons learned. Mayo Clin Proc 2005;80(11):1449–1460. DOI: 10.4065/80.11.1449.
11. Perry JE, Churchill LR, Kirshner HS. The Terri Schiavo case: Legal, ethical, and medical perspectives. Ann Intern Med 2005;143(10):744–748. DOI: 10.7326/0003-4819-143-10-200511150-00012.
12. Zhou JY, Amanatullah DF, Frick SL. EMTALA (Emergency Medical Treatment and Active Labor Act) obligations: A case report and review of the literature. J Bone Joint Surg Am 2019;101(12). DOI: 10.2106/JBJS.18.01166.
13. Zuabi N, Weiss LD, Langdorf MI. Emergency Medical Treatment and Labor Act (EMTALA) 2002–15: Review of Office of Inspector General patient dumping settlements. West J Emerg Med 2016;17(3):245–251. DOI: 10.5811/westjem.2016.3.29705.

14. Lulla A, Svancarek B. EMS USA emergency medical treatment and active labor act. StatPearls. Accessed on: December 7, 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK539798/.
15. House of Representatives C. 42 U.S.C. 1395dd: Examination and treatment for emergency medical conditions and women in labor. govinfo.gov. Accessed on: December 7, 2025. Available from: https://www.govinfo.gov/app/details/USCODE-2010-title42/USCODE-2010-title42-chap7-subchapXVIII-partE-sec1395dd.
16. Warby R, Leslie SW, Borger J. EMTALA and patient transfers. StatPearls. Accessed on: December 7, 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK557812/.
17. Understanding EMTALA. ACEP. Accessed on: December 7, 2025. Available from: https://www.acep.org/life-as-a-physician/ethics--legal/emtala/emtala-fact-sheet.
18. Voultsos P, Chatzinikolaou F. Involuntary euthanasia of severely ill newborns: Is the Groningen Protocol really dangerous? Hippokratia 2014;18(3):193. Accessed on: December 7, 2025. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4309136/.
19. Vizcarrondo FE. Neonatal euthanasia: The Groningen Protocol. Linacre Q 2014;81(4):388. DOI: 10.1179/0024363914Z.00000000086.
20. Verhagen E, Sauer PJJ. The Groningen Protocol: Euthanasia in severely ill newborns. N Engl J Med 2005;352(10):959–962. DOI: 10.1056/NEJMP058026.
21. Survival and outcomes for the extremely preterm: The NICHD network results continue to improve. Neonatal Research. Accessed on: December 9, 2025. Available from: https://neonatalresearch.org/2022/01/20/survival-and-outcomes-for-the-extremely-preterm-the-nichd-network-results-continue-to-improve-can-we-do-even-better/.
22. Extremely preterm birth outcomes tool. NICHD. Accessed on: December 9, 2025. Available from: https://www.nichd.nih.gov/research/supported/EPBO.
23. Patel RM. Short and long-term outcomes for extremely preterm infants. Am J Perinatol 2016;33(3):318. DOI: 10.1055/S-0035-1571202.
24. Younge N, Goldstein RF, Bann CM, et al. Survival and neurodevelopmental outcomes among periviable infants. N Engl J Med 2017;376(7):617–628. DOI: 10.1056/NEJMOA1605566.
25. Serenius F, Ewald U, Farooqi A, et al. Neurodevelopmental outcomes among extremely preterm infants 6.5 years after active perinatal care in Sweden. JAMA Pediatr 2016;170(10):954–963. DOI: 10.1001/JAMAPEDIATRICS.2016.1210.
26. Vanhaesebrouck P, Allegaert K, Bottu J, et al. The EPIBEL study: Outcomes to discharge from hospital for extremely preterm infants in Belgium. Pediatrics 2004;114(3):663–675. DOI: 10.1542/PEDS.2003-0903-L.
27. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD neonatal research network. Pediatrics 2010;126(3):443–456. DOI: 10.1542/PEDS.2009-2959.
28. Costeloe KL, Hennessy EM, Haider S, et al. Short term outcomes after extreme preterm birth in England: Comparison of two birth cohorts in 1995 and 2006 (the EPICure studies). BMJ 2012;345(7886). DOI: 10.1136/BMJ.E7976.
29. Pignotti MS, Berni R. Extremely preterm births: End-of-life decisions in European countries. Arch Dis Child Fetal Neonatal Ed 2010;95(4). DOI: 10.1136/ADC.2009.168294.
30. MacLean G, Gallipoli A, Raabis A. 23 current trends in Canada in neonatal palliative care. Paediatr Child Health 2020;25(Suppl 2):e8. DOI: 10.1093/PCH/PXAA068.022.
31. Akyempon AN, Aladangady N. Neonatal and perinatal palliative care pathway: A tertiary neonatal unit approach. BMJ Paediatr Open 2021;5(1). DOI: 10.1136/BMJPO-2020-000820.
32. Canadian Network of Palliative Care for Children. CHPCA. Accessed on: December 9, 2025. Available from: https://www.chpca.ca/initiatives/canadian-network-of-palliative-care-for-children/.
33. Harnden F, Lanoue J, Modi N, et al. Data-driven approach to understanding neonatal palliative care needs in England and Wales: A population-based study 2015–2020. Arch Dis Child Fetal Neonatal Ed 2023;108(5):540–544. DOI: 10.1136/ARCHDISCHILD-2022-325157.
34. Wilkinson D, Bertaud S, Mancini A, et al. Recognising uncertainty: An integrated framework for palliative care in perinatal medicine. Arch Dis Child Fetal Neonatal Ed 2025;110(3):F236–F244. DOI: 10.1136/ARCHDISCHILD-2024-327662.
35. New BAPM framework for palliative care in perinatal medicine. British Maternal & Fetal Medicine Society. Accessed on: December 9, 2025. Available from: https://www.bmfms.org.uk/professionals/news/206/new_bapm_framework_for_palliative_care_in_perinatal_medicine/.
36. Palliative care: A framework for clinical practice in perinatal medicine. British Association of Perinatal Medicine. Accessed on: December 9, 2025. Available from: https://www.bapm.org/resources/30-palliative-care-a-framework-for-clinical-practice-in-perinatal-medicine-2010.
37. Perinatal palliative care: ACOG committee opinion number 786. Obstet Gynecol 2019;134(3):E84–E89. DOI: 10.1097/AOG.0000000000003425.
38. Lee JH, Lee SY, Cha KM. An evolutionary concept analysis of pediatric hospice and palliative care. J Hosp Palliat Care 2024;27(2):51. DOI: 10.14475/JHPC.2024.27.2.51.
39. World Health Organisation. Human genome editing: A framework for governance. 2021;26–28.
40. Xue Y, Shang L. Governance of heritable human gene editing worldwide and beyond. Int J Environ Res Public Health 2022;19(11). DOI: 10.3390/IJERPH19116739.
41. Cadigan RJ, Waltz M, Conley JM, et al. Human heritable genome editing and its governance: Views of scientists and governance professionals. New Genet Soc 2024;43(1):e2404061. DOI: 10.1080/14636778.2024.2404061.
42. Raposo VL. The first Chinese edited babies: A leap of faith in science. JBRA Assist Reprod 2019;23(3):197. DOI: 10.5935/1518-0557.20190042.
43. Liu Z, Shi J, Xu J. He Jiankui's unprecedented offense and worrying comeback: How scandals reshape the legal governance of scientific research in China? J Responsible Innov 2024;11(1). DOI: 10.1080/23299460.2024.2372116.
44. Greely HT. CRISPR'd babies: Human germline genome editing in the 'He Jiankui affair'. J Law Biosci 2019;6(1):111. DOI: 10.1093/JLB/LSZ010.
45. National Academies of Sciences, Engineering, and Medicine; National Academy of Medicine; National Academy of Sciences; Committee on Human Gene Editing: Scientific, Medical, and Ethical Considerations. Human genome editing: Science, ethics, and governance. 2017:1–310. DOI: 10.17226/24623.
46. Drabiak K. The Nuffield Council's green light for genome editing human embryos defies fundamental human rights law. Bioethics 2020;34(3):223–227. DOI: 10.1111/BIOE.12713.
47. Hawkes N. Human genome editing is not unethical, says Nuffield Council. BMJ 2018;362:k3140. DOI: 10.1136/BMJ.K3140.
48. Genome editing and human reproduction: Social and ethical issues. Nuffield Council on Bioethics. Accessed on: December 9, 2025. Available from: https://www.nuffieldbioethics.org/publication/genome-editing-and-human-reproduction-social-and-ethical-issues/.
49. The Lancet. Charlie Gard and the limits of medicine. Lancet 2017;390(10094):531. DOI: 10.1016/S0140-6736(17)32159-1.
50. Wilkinson D, Savulescu J. The Charlie Gard case. Accessed on: December 9, 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537990/.
51. Wilkinson D, Savulescu J. Hard lessons: Learning from the Charlie Gard case. J Med Ethics 2018;44(7):438–442. DOI: 10.1136/MEDETHICS-2017-104492.
52. Paris JJ, Ahluwalia J, Cummings BM, et al. The Charlie Gard case: British and American approaches to court resolution of disputes over medical decisions. J Perinatol 2017;37(12):1268. DOI: 10.1038/JP.2017.138.
53. Dyer C. Law, ethics, and emotion: The Charlie Gard case. BMJ 2017;358. DOI: 10.1136/BMJ.J3152.
54. Łaszewska-Hellriegel M. The cases of Alfie Evans and Charlie Gard: Who should decide when to end a therapy? Ethique Sante 2019;16(2):71–80. DOI: 10.1016/J.ETIQE.2019.03.003.
55. Lantos JD, Feudtner C. SUPPORT and the ethics of study implementation: Lessons for comparative effectiveness research from the trial of oxygen therapy for premature babies. Hastings Cent Rep 2014;45(1):30. DOI: 10.1002/HAST.407.

56. Lantos JD. SUPPORTing premature infants. Pediatrics 2013;132(6):e1661. DOI: 10.1542/PEDS.2013-1292.
57. Singer P. How not to save a life. Bioethics 2011;25(5):ii–iii. DOI: 10.1111/J.1467-8519.2011.01906.X.
58. "Baby Joseph" dies at home after long treatment battle. ABC News. Accessed on: December 9, 2025. Available from: https://abcnews.go.com/Health/Wellness/baby-joseph-dies-home-long-treatment-battle/story?id=14623722.

REVIEW ARTICLE

Maternal Determinants of Neurodiversity in the Developing Brain: An Integrative Review of Biological, Environmental, and Psychological Factors

Chhaya S Prasad[1–3], Akhil Maheshwari[3–19]

Received on: 20 October 2025; *Accepted on*: 23 December 2025; *Published on*: 15 January 2026

Abstract

Neurodevelopmental delay is a major concern for obstetricians, neonatologists, and pediatricians all over the world. However, more recently, a view is emerging that we need to be cognizant of neurodiversity, a natural variation in cognitive and behavioral traits observed across individuals. Many differences in the human brain originate in the earliest developmental periods, when maternal biological signals and environmental conditions shape the fetal neural architecture. These temporal windows prior to conception, during embryogenesis, and fetal development are marked by significant plasticity and possibly shape lifelong neurodevelopment. The structural and functional changes can be affected by maternal metabolic, endocrine, immune, and psychosocial states, which act through the placenta to influence neurogenesis, synaptogenesis, and the assembly of large-scale networks. Differences in thinking, learning, attention, and behavior are a normal part of human diversity. Hence, conditions such as autism, attention-deficit and hyperactivity disorder (ADHD), and dyslexia might not be true deficits but alternative ways of experiencing and interacting with the outside world. The acceptance of these special needs as neurodiversity, not neurological deficits, promotes acceptance, inclusion, and support, and encourages societies, schools, and workplaces to value diverse cognitive strengths while providing appropriate accommodations for individual needs.

Keywords: 11β-hydroxysteroid dehydrogenase type II, Arsenic, Attention-deficit and hyperactivity disorder, Autism, Cortical layering, Dendritic arborization, Developmental origins of neurodevelopment, DNA methylation, Dyslexia, Epigenetic clock, Epigenetic drift, Epigenetic programming, Fetal signaling, Gamete quality, Heavy metals, Histone modifications, Infant, Interleukin-17A, Intimate partner violence, Ionizing medical radiation, Lead, Legal liability, Leptin signaling, Low-frequency electromagnetic fields, Maternal biological signals, Methylmercury exposure, Microbial seeding, Microglial maturation, Microglial priming, Mitochondrial DNA, Network topology, Neural progenitor proliferation, Neurodevelopmental disorders, Neurodevelopmental trajectories, Neuronal migration, Nitrogen dioxide, Non-coding RNA, Non-ionizing radiofrequency, Omega-3-long-chain polyunsaturated fatty acids, Organophosphate pesticides, Periconceptional folate, Phthalates, Plastics, PM10, PM2.5, Polybrominated diphenyl ethers, Synaptic pruning, Synaptogenesis, Tryptophan–kynurenine pathway, White matter scaffolding.

Newborn (2025): 10.5005/jp-journals-11002-0147

Key Points

- Neurodevelopmental delay is a major concern for obstetricians, neonatologists, and pediatricians all over the world. However, more recently, a view is emerging that this structural/functional variation in the developing brain might need to be viewed as neurodiversity, a natural variation in cognitive and behavioral traits in a population.
- Neurological development can be altered in the earliest developmental periods beginning prior to conception, during embryogenesis, and in the developing fetus, when maternal biological signals and environmental conditions shape the fetal neural architecture. These temporal windows are marked by significant plasticity.
- The concept of neurodiversity suggests that differences in thinking, learning, attention, and behavior show a normal spectrum of our brain function. Hence, conditions such as autism, attention-deficit/hyperactivity disorder (ADHD), and dyslexia might not be true deficits but alternative ways of experiencing and interacting with the outside world.
- The acceptance of these special needs as neurodiversity, not neurological deficits, promotes acceptance, inclusion, and support, and encourages societies, schools, and workplaces to value diverse cognitive strengths while providing appropriate accommodations for individual needs.

[1]Developmental Pediatrics, ASHA Center for Child Development and Scientific Research, Chandigarh, India

[2]Autism Care Network Foundation, Chandigarh, India

[3]Global Newborn Society, Harrison, New York, United States of America

[4]Department of Pediatrics/Neonatology, Boston Children's Health Physicians Group at the Maria Fareri Children's Hospital, New York Medical College, Valhalla, New York, United States of America

[5]Banaras Hindu University Institute of Excellence, Varanasi, Uttar Pradesh, India

[6]Mongolian Association of Obstetrics, Gynecology, and Neonatology, Ulaanbaatar, Mongolia

[7]S.A.B.R.E.E. Enrichment Academy, Saint Louis, Missouri, United States of America

[8]The Skylar Project, Daphne, Alabama, United States of America

[9]American Society for Marginalized Lives, Harrison, New York, United States of America

[10]PreemieWorld Foundation, Springfield, Virginia, United States of America

[11]Carlo GNS Center for Saving Lives at Birth, Birmingham, Alabama, United States of America

[12]GNS Forum for Transgenerational Inheritance, New York, United States of America

- In this article, we present maternal factors known to be associated with neurodiversity in the developing brain.

Introduction

The central nervous system grows rapidly during the gestational period and after birth; anatomic growth can be seen in both size/volume and in the surface area (formation of gyri/sulci; Fig. 1). Unfortunately, functional neurodevelopmental delay is seen not-so-infrequently and is a major concern for obstetricians, neonatologists, and pediatricians all over the world. In recent times, a view is emerging that we need to be cognizant of neurodiversity, a natural variation in cognitive and behavioral traits observed across individuals. This recognition of diversity carries a view that a wide range of exposures during early development, ranging from maternal/fetal cytokines, hormones such as glucocorticoids, micronutrients, and environmental toxicants, can alter neurological maturation and even the eventual outcomes in health and disease.[1–22] Population studies link maternal diabetes, obesity, infection/inflammation, nutrient deficiencies, stress, and exposure to air pollution and endocrine-disrupting chemicals with learning disabilities, autism-spectrum conditions, attention-deficit and hyperactivity disorder (ADHD), language delay, and emotional dysregulation.[2,9,10,15–17,23–38]

Definitions and Concepts

Neurodiversity describes the range of human differences in attention, communication, sensory processing, and social cognition as natural variation rather than simple deficits.[1,39–41] Population-level heterogeneity in traits and diagnoses reflects interactions among polygenic architecture, early developmental biology, and environment across time.[11,42] At the system level, case–control and dimensional imaging studies report differences in functional connectivity and microstructural organization across association cortices, salience and default networks, and fronto-striatal circuits.[43] These differences often reflect timing and calibration of development rather than injury.[38,44]

Developmental Origins of Neurodevelopment (DOHaD)

The DOHaD perspective places exceptional weight on the periconceptional period and gestation, when epigenetic programming is highly labile and small signals can have durable effects on brain structure and function.[45–47] DNA methylation, histone modifications, and non-coding RNAs regulate gene expression in progenitor pools and maturing neurons, influencing migration, dendritic arborization, and synaptic pruning.[48–50] The placenta functions as an immune, endocrine, transport, and sensing organ that transduces maternal cues, including inflammation, glucocorticoids, micronutrients, and xenobiotics, into fetal signals that recalibrate developmental set points.[25]

Importance of Maternal Factors

For the fetus, the maternal environment is *the* environment. Maternal metabolic, immune, endocrine, and psychosocial states shape the biochemical landscape in which the brain's architecture is assembled.[25,51] A substantial proportion of cortical layering, white matter scaffolding, and large-scale network topology is established before birth, with later refinement through experience-dependent plasticity.[44,52] Common maternal conditions such as diabetes, obesity, thyroid dysfunction, infection, stress, malnutrition, and environmental toxicant exposure operate during critical windows

[13]Bangladesh Neonatal Foundation, Dhaka, Bangladesh

[14]Dr. Mozib Newborn Foundation, Dhaka, Bangladesh

[15]Neonatology-Certified Foundation, Brooksville, Texas, United States of America

[16]GNS Infant Nutrition Education Program, Harrison, New York, United States of America

[17]Pioneers–Looking for sustainable ways to reduce infant mortality, Oslo, Norway

[18]International Prader-Willi Syndrome Organization, Cambridge, United Kingdom

[19]First Breath of Life, Shreveport, Louisiana, United States of America

Corresponding Author: Chhaya S Prasad, Developmental Pediatrics, ASHA Center for Child Development and Scientific Research, Chandigarh, India; Autism Care Network Foundation, India; Global Newborn Society, Harrison, New York, United States of America, Phone: +91 9876819949, e-mail: chhaya.sam@gmail.com

How to cite this article: Prasad CS, Maheshwari A. Maternal Determinants of Neurodiversity in the Developing Brain: An Integrative Review of Biological, Environmental, and Psychological Factors. Newborn 2025;4(4):203–220.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as the Editor-in-Chief of this journal and this manuscript was subjected to this journal's standard review procedures, with this peer review handled independently of Editor-in-Chief and his research group.

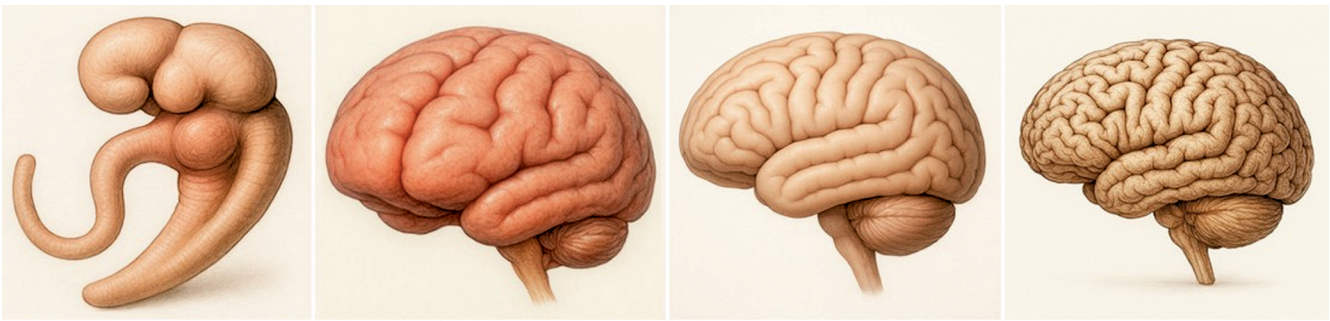


Fig. 1: Graphical illustrations of the developing brain. The increasing complexity of the gyri and sulci can be seen. Neurodiversity can arise in structural and/or functional alterations during development

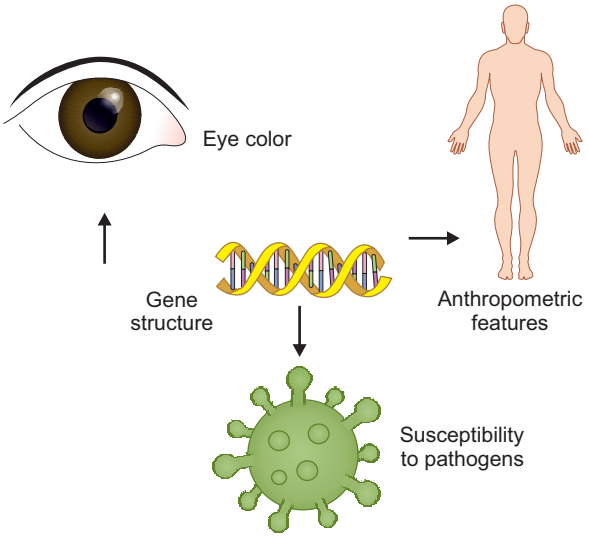


Fig. 2: Pleiotropy: Variation in the structure of a single gene can influence seemingly unrelated traits. These changes can arise in various components/regulators of a gene, including enzymes, hormones, or structural component(s), which affect multiple biological pathways or processes. Hence, a mutation in that one gene can have wide-ranging effects on an organism's phenotype

when even modest perturbations can shift neurodevelopmental trajectories.[17,51,53] Prevention and early identification, therefore, begin well before delivery.

Maternal Factors that Influence Neurodiversity

Neurological development reflects the integration of maternal genetic architecture, epigenetic programming, physiology, behavior, and the social context with the fetal and placental *milieu*.[54–56] Each factor can be modest in isolation, yet the effect can be cumulative and interactive during sensitive periods.

Maternal Genetic and Epigenetic Factors

Maternal factors have been linked with the risk of autism and ADHD through genetic/epigenetic and other effects that shape the intrauterine environment, which influence immune tone, metabolism, or hormone levels.[1,57–59] Polygenic risk scores for neurodevelopmental conditions partly overlap with scores for metabolic traits and immune traits, which suggests pleiotropy (Fig. 2) at the maternal level that can alter placental function and fetal growth patterns.[60–63]

Epigenetic inheritance is most relevant around conception and early implantation when DNA methylation is being extensively reestablished.[64–66] Maternal nutritional state, inflammation, and endocrine milieu can leave durable methylation and histone marks in fetal tissues, including the placenta, with downstream effects on neurogenic niches and synaptogenesis.[25,67]

Maternal age can alter the epigenetic clock and gamete quality, which may influence chromosomal integrity and early patterning.[2,68,69] Studies linking maternal age to neurodevelopmental outcomes likely reflect a mixture of *de novo* mutation risk, epigenetic drift, and social factors; therefore, effect sizes are context dependent.[23,70,71] Alterations in mitochondrial DNA, being maternally inherited, have been noted in some children with neurodevelopmental diagnoses; maternal mitochondrial variants or damaged segments can affect fetal energy metabolism and

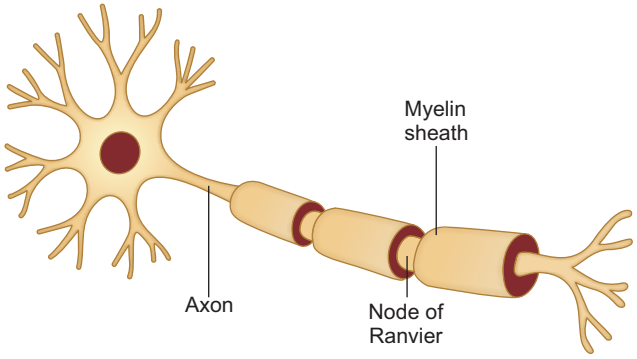


Fig. 3: Fetal nerve myelination is a developmental process in which myelin, a fatty insulating layer, forms around nerve fibers to improve the speed and efficiency of signal transmission. It is largely composed of Schwann cells and shows intermittent gaps known as the nodes of Ranvier. Myelination is a key variable for normal motor control, sensory processing, and cognitive development. This process begins during late pregnancy and continues after birth, following a carefully regulated sequence that supports the maturation of the nervous system

myelination (Fig. 3), although population-level estimation needs work.[72–74]

X-linked variants contribute to sex differences in risk of altered neurodevelopment.[73,75] Hemizygosity in male offspring increases penetrance for certain loci, and placental and immune dimorphism by fetal sex may further modulate vulnerability to maternal signals.[11]

Maternal Metabolic Conditions

Maternal obesity, insulin resistance, and diabetes are associated with a higher risk of autism, ADHD traits, and developmental delay in offspring in meta-analyses and large cohorts, with risk gradients that track glycemic control and body mass index categories.[29,76–78] Probable mechanisms include chronic low-grade inflammation, oxidative stress, dysregulated lipid mediators, altered insulin and leptin signaling to the brain, and placental vascular and transport changes that affect nutrient timing and availability.[21,78]

Hyperglycemia and hyperinsulinemia can influence neural progenitor proliferation and differentiation, microglial priming, and later myelination.[78,79] Maternal dyslipidemia and fatty acid imbalance can alter membrane composition and eicosanoid profiles, which may affect synaptic function.[80,81] Interventions that improve glycemic control, weight gain within guidelines, and dietary quality are associated with better obstetric outcomes, though more definitive neurodevelopmental prevention trials are needed.[82,83]

Maternal Endocrine Factors

The thyroid hormone is critical for neuronal migration, cerebellar development, and myelination.[84,85] Associations between maternal hypothyroidism or hypothyroxinemia and adverse cognitive or behavioral outcomes are consistent across cohorts, although confounding and timing of treatment influence effect estimates.[86]

Ensuring iodine sufficiency remains a low-cost prevention strategy in regions with marginal intake.[87] Polycystic ovary syndrome combines hyperandrogenism, insulin resistance, and inflammatory signaling.[88] Registry studies inconsistently report higher rates of neuropsychiatric outcomes in offspring of mothers with specific conditions such as polycystic ovary syndrome, although shared familial factors likely contribute, and absolute risks remain small.[4,12,15,18,23,30,32,33,89,90]

Maternal stress activates the hypothalamic-pituitary-adrenal (HPA) axis and increases fetal glucocorticoid exposure through placental transfer and local enzyme changes.[91] This can modify amygdala and hippocampal development, stress reactivity, and sleep regulation, with downstream effects on attention and emotional regulation.[22,92]

Maternal Immune Activation and Inflammation

Maternal immune activation can range from chronic low-grade inflammation to discrete febrile infections and clinically defined autoimmune disease.[93] Low-grade inflammation, often driven by obesity, insulin resistance, periodontal disease, environmental exposures, poor sleep, or sustained psychosocial stress, raises basal cytokine tone and alters placental endocrine and vascular function.[94] The placenta responds to this milieu by adjusting glucocorticoid metabolism, tryptophan and serotonin handling, and nutrient transport, which in turn changes fetal signaling set points during periods when neuronal migration, synaptogenesis, and microglial maturation are highly sensitive.[17,25,95] Prospective data that use inflammatory biomarkers support this link. In a national birth cohort, higher maternal C-reactive protein in early pregnancy was associated with greater odds of autism in offspring, which is consistent with a dose-related contribution of background inflammation, although the absolute risk increase at the individual level is small.[96,97]

- Autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, and autoimmune thyroiditis provide a clinically traceable model of sustained maternal immune activity.[93] Meta-analytic evidence indicates increased odds of autism in offspring of mothers with autoimmune disease, with variability by disease and timing, and likely contributions from shared genetic architecture and treatment effects.[31] Thyroid autoimmunity is a special case because it can coexist with subtle thyroid hormone insufficiency, thereby converging endocrine and immune mechanisms that influence neuronal migration and myelination.[98] The clinical implication is straightforward. Optimizing disease control and maintaining euthyroidism during pregnancy are sensible targets that may limit prolonged cytokine exposure to the placenta and fetus.[99]
- Infections during pregnancy add episodic immune surges on top of background immune tone.[100] Large register-based studies report that maternal viral/bacterial infections requiring medical attention, and fever in particular, are associated with higher rates of autism or developmental delay, with effect estimates that vary by gestational timing and pathogen class.[30,101] Historical and serologically documented cohorts extend the spectrum to adult outcomes, where mid-gestation influenza exposure has been linked to increased schizophrenia risk, again with small absolute effects but consistent biological plausibility.[96]

Mechanisms

These population signals fit well with mechanistic work showing that specific cytokines are sufficient to alter developmental programs.

- Cytokine-driven recalibration of neurodevelopmental processes in a time-dependent manner is a shared theme.[102] Interleukin-6 (IL-6) acts as a key mediator that couples maternal inflammation to atypical cortical development and later behavioral phenotypes in offspring.[103] Interleukin-17A, arising from a maternal T-helper 17 (Th-17) skew, produces focal cortical dysplasia and social and sensory phenotypes in mice, an effect that depends on maternal gut microbial communities that promote Th-17 differentiation.[3,104–106] Tumor necrosis factor (TNF) participates in the same network by modifying placental perfusion, endothelial integrity, and microglial activation within the fetal brain, thereby shifting synaptic formation and pruning trajectories.[17]
- Epidemiological evidence: The strongest and most consistent epidemiological links fall on the autism and schizophrenia spectra, which likely reflects the convergence of immune-mediated effects on cortical circuit assembly, synaptic refinement, and stress response calibration during mid-fetal development.[107] Sex-specific placental responses and the steroid *milieu* may amplify vulnerability in male fetuses, while gene and environment interplay probably explains why seeming similar immune exposures lead to different outcomes across families.[13,22] For clinical practice, the message is proportionate and actionable. Vaccination according to obstetric guidance, prompt evaluation and treatment of infection, fever control, tight control of autoimmune disease, sleep and nutrition support, and reduction of modifiable inflammatory drivers are rational steps that can lower cumulative cytokine exposure without generating undue alarm for individual pregnancies.
- Oxidative stress acts in parallel. Hypoxia–reoxygenation at the maternal–placental interface, metabolic dysfunction, and pollutant exposures generate reactive oxygen species that damage lipids and DNA, impair endothelial function, and further amplify cytokine signaling.[108,109] These processes remodel placental perfusion and barrier characteristics and can alter neuronal proliferation and oligodendrocyte maturation during periods when white matter tracts are forming.[25]
- Diet and metabolic status also condition inflammatory tone, creating fertile ground for interaction with other risks.[10,17,21,28,89,104,110,111] In pregnant women with overweight or obesity, low-grade inflammation is common and can be attenuated by higher omega-3 intake, which reduces placental and adipose expression of inflammatory mediators such as TLR4, IL-6, IL-8, and TNF and lowers C-reactive protein.[89] By the same logic, proinflammatory dietary patterns or insulin resistance may magnify the neurodevelopmental effects of infections, pollutants, or psychosocial stress that operate through the same cytokine networks.[112] Experimental work supports this convergence: Maternal high-fat diet reshapes the gut microbiome and impairs offspring social behavior through synaptic and oxytocinergic pathways, changes that are reversible with microbial reconstitution, underscoring how nutrition-immune-microbiome axes modulate neurobehavioral outcomes.[105]

The gut–brain axis links maternal diet, microbiome, and fetal neuroimmune development (Fig. 4).[113] Maternal dietary patterns reshape microbial communities and metabolite profiles.[114] Experimental work indicates that specific microbial metabolites influence microglial maturation and thalamocortical development, providing a route from maternal nutrition and dysbiosis to circuit formation in the fetus.[3,106]

Maternal Nutrition

Deficiencies of Specific Nutrients

Periconceptional folate supplementation can help prevent neural tube defects and has also been associated with lower autism risk

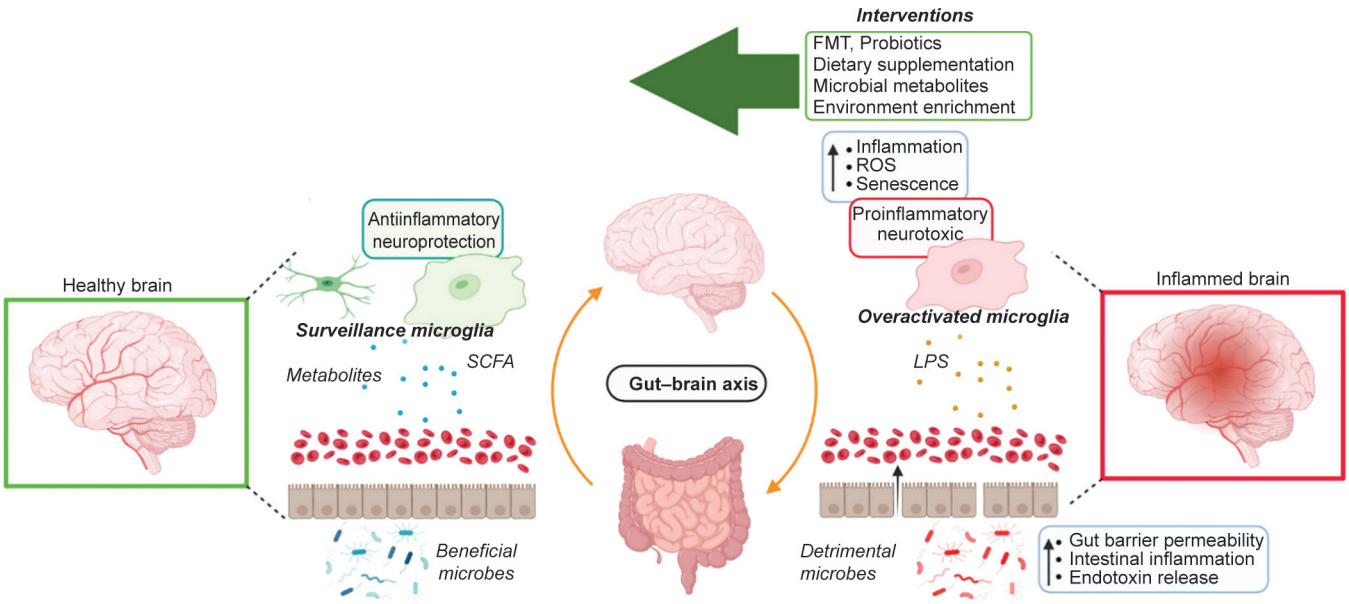


Fig. 4: Gut–brain axis describes the communication between the gut, immune system, and nervous system, which is likely to be particularly important during pregnancy and lactation. A mother's diet shapes the composition of her gut microbiome, which in turn can produce metabolites such as short-chain fatty acids that can influence her immune responses in the brain (as indicated in microglial activation). These immune signals and metabolites can cross the placenta or affect placental function, helping to guide fetal brain development and immune system maturation. Disruptions to the maternal microbiome, caused by poor diet, inflammation, or environmental exposures, may alter this signaling, potentially influencing neurodevelopmental outcomes in the fetus. FMT, fecal microbiota transfer; LPS, lipopolysaccharide; SCFA, short-chain fatty acids

in several cohorts, consistent with roles in one-carbon metabolism, methylation, and nucleotide synthesis.[34,115]

Iron is needed for mitochondrial enzymes, neurotransmitter synthesis, and myelination. Maternal iron deficiency associates with cognitive delay and behavior problems, and the timing also appears to be important because oligodendrocyte maturation is sensitive to iron availability.[24]

Low maternal vitamin D has been linked to autism related traits and language delay in observational studies.[116] Vitamin D may influence neurotrophins, calcium signaling, and immune regulation, yet trial data for neurodevelopmental endpoints remain limited and heterogeneous.[7,24,115]

Omega-3 long-chain polyunsaturated fatty acids contribute to membrane fluidity, synaptic function, and antiinflammatory signaling.[117] Meta-analyses suggest small improvements in attention and behavior with supplementation in selected groups, with effect modification by baseline diet and dose.[111]

High Fat and Sugar Intake

High-fat and sugar dietary patterns increase insulin resistance, oxidative stress, and proinflammatory cytokines, and they shift the maternal microbiome toward less favorable profiles.[118] These changes can influence placental function and fetal brain development in animal models and human cohorts with dietary pattern scoring, although disentangling diet from broader social factors is challenging.[17] Diets rich in ultra-processed foods and *trans* fatty acids may worsen endothelial function and oxidative stress and are plausibly harmful during pregnancy.[119]

Specific Nutritional Patterns

Maternal diet shapes the gut microbiome and metabolite profiles. Experimental work shows that microbial metabolites can regulate microglial maturation and thalamocortical development, which provides a plausible route linking maternal dietary quality to fetal neurodevelopment.[106] In humans, associations between dietary patterns, microbiome composition, and child neurodevelopment are emerging but require longitudinal confirmation.[120]

Maternal Mental Health

- Maternal depression, anxiety, and post-traumatic stress symptoms in pregnancy have been noted frequently across cohorts and in meta-reviews; antenatal mood and anxiety are associated with higher rates of attentional difficulties, emotional and behavioral problems, and language delays in the offspring(s), even after accounting for socioeconomic factors and parental education.[121,122] Perinatal mood disorders that begin in pregnancy or peak in the early postpartum period often follow a relapsing course and can co-occur with sleep disruption, reduced nutritional quality, and elevated inflammatory markers, which together sustain biological signals that reach the placenta and fetus.[121]
- Chronic stress and toxic stress reflect cumulative adversity that exceeds a family's regulatory capacity. These exposures are linked to persistent activation of the maternal HPA axis, higher circulating cortisol, and altered diurnal rhythms.[13] The placenta modulates glucocorticoid transfer to the fetus through 11β-hydroxysteroid dehydrogenase type II and transporter systems. Under chronic stress, inflammatory signaling and reduced placental 11β-hydroxysteroid dehydrogenase type II activity can increase fetal glucocorticoid exposure, while cytokine and tryptophan–kynurenine pathway shifts may influence neuronal migration, dendritic arborization, and microglial maturation.[123] These processes converge on limbic and fronto-striatal circuits that support arousal regulation, executive function, and language acquisition, creating plausible routes from maternal symptoms to child outcomes.[13,121,124]

- Antenatal anxiety and perceived stress associate with greater ADHD symptom burden and attentional control difficulties in preschool and school years, with effect sizes that rise when exposure occurs in mid to late gestation and when stress is prolonged.[13,124] Emotional dysregulation and externalizing behaviors are more frequent when maternal depression is persistent from pregnancy into the postpartum period, suggesting combined intrauterine and caregiving pathway effects; targeted treatment and psychosocial support reduce these risks.[121,122] Natural experiment data from disaster exposures show that objectively measured prenatal stress relates to language and cognitive outcomes, which underscores the importance of timing and intensity; children exposed in mid-gestation tend to show more pronounced language delays and attentional problems.[125] Post-traumatic stress symptoms in pregnancy, particularly when associated with sleep loss and comorbid depression, are linked to greater behavioral problems and stress reactivity in early childhood, again with small to moderate effects at the population-level and substantial heterogeneity by support, treatment, and postnatal environment.[13,121]

Clinically, a proportional response is warranted.[126] Screening for antenatal depression, anxiety, and trauma exposure using brief validated tools, followed by timely psychological and, when indicated, pharmacologic treatment, is associated with improved maternal functioning and may reduce child risk.[121] Counseling to improve sleep, nutrition quality, and social support can dampen basal inflammation and normalize diurnal cortisol patterns. Communication with families should be balanced and specific. Most children do well, absolute risks for any one symptom domain are small, and supportive prenatal care plus early parenting support are meaningful levers that can shift trajectories toward better attention, emotional regulation, language, and behavior.

Maternal Environmental Exposures

Maternal environmental exposures form a shifting background of air pollutants, metals, and endocrine-disrupting chemicals that co-occur with social stressors and can cumulatively influence fetal brain development.[14] The placenta senses this composite signal and translates it through oxidative stress, endothelial dysfunction, and endocrine perturbation, with vulnerability varying by gestational window.[8,25]

Physical Exposures

Ambient air pollution remains one of the most pervasive prenatal exposures.[127] Fine and coarse particulate matter and traffic-related gases such as PM2.5, PM10, and nitrogen dioxide cross or signal across the placenta and are linked to systemic and placental oxidative stress, endothelial dysfunction, and low-grade inflammation.[128,129] These processes can alter uteroplacental blood flow, trophoblast endocrine output, and transporter expression that together shape fetal brain growth and network assembly.[130] Cohort studies associate higher prenatal exposure to traffic-related pollution and particulate matter with greater odds of autism-spectrum diagnoses and with lower cognitive scores, with susceptibility that appears strongest in mid to late gestation and in settings with combined social adversity.[8,35,131]

Heavy metals are established developmental neurotoxicants and several retain relevance in pregnancy.[132] Lead readily crosses the placenta from maternal bone and blood stores.[133] Even low-level exposure *in utero* and infancy correlates with lower developmental quotients and attentional problems in childhood, and there is no known safe threshold for the developing brain (Fig. 5).

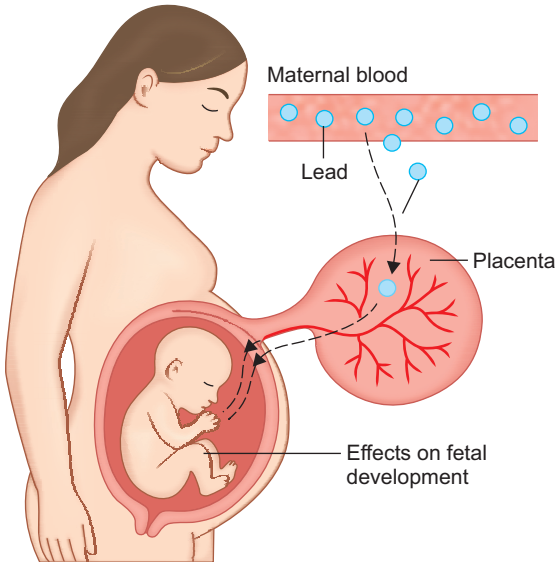


Fig. 5: Fetal lead poisoning occurs when lead crosses from the maternal tissues/blood into the developing fetus. The fetal nervous system is highly sensitive, and even low levels of lead exposure can result in serious consequences such as developmental delay, learning difficulties, low birth weight, premature birth, and behavioral problems later in life. Lead stored in maternal bones from past exposure can also be released into the bloodstream during pregnancy, increasing risk even without current exposure

Methylmercury exposure is mainly dietary and varies by fish species and geography.[134] Prenatal exposure to methylmercury is associated with language and memory deficits and with poorer visual attention, while maternal consumption of low mercury fish that are rich in omega-3 fatty acids can be neuroprotective, which underscores the need for species-specific counseling rather than blanket avoidance.[8,135] Arsenic exposure, typically from contaminated groundwater or rice-based foods, has been linked to lower cognitive performance and behavioral problems in cohort studies from exposed regions.[136] Proposed mechanisms include interference with neurogenesis and synaptogenesis, mitochondrial dysfunction, and endocrine disruption, with dose and timing shaping the magnitude of effect.[8,137]

Plastics and related additives are seen frequently in food contact materials and consumer products, and several act as endocrine-disrupting chemicals (Fig. 6). Bisphenol A, a synthetic chemical commonly used to make certain plastics, can interact with estrogen and thyroid signaling and has been associated with differences in executive function and externalizing behavior following prenatal exposure, although measurement error and confounding remain concerns in human studies.[138]

Several types of phthalates (Fig. 7), which are used as plasticizers and in personal care products, are linked to attention problems and social behaviors in multiple cohorts. Proposed pathways include altered androgen and thyroid signaling, oxidative stress, and epigenetic changes in the placenta and fetal brain.[139]

Ionizing medical radiation is fortunately uncommon in pregnancy and, at diagnostic doses, rarely approaches thresholds associated with deterministic neurodevelopmental injury. Large

guidance documents and cohort data indicate that most single diagnostic studies deliver fetal doses well below levels linked to microcephaly or intellectual disability, which are mainly observed after exposures above about 100 mGy during weeks 8 to 15 (ACOG Committee Opinion, 2017).[140] When imaging is clinically necessary, ultrasound or magnetic resonance imaging without contrast is preferred; population data show no increase in stillbirth, congenital anomalies, neoplasia, vision or hearing loss, or neurodevelopmental deficits after non-contrast MRI at any gestational age, although gadolinium exposure has been associated with higher rates of rheumatologic or inflammatory skin conditions and stillbirth or neonatal death in one large cohort.[141] Most clinicians therefore avoid non-urgent ionizing studies to individualize risk and benefit when CT is considered and to avoid gadolinium unless a compelling indication exists.

Non-ionizing radiofrequency and extremely low-frequency electromagnetic fields (EMFs) from mobile phones, Wi-Fi, and power sources have been investigated for potential effects on child behavior and cognition. Several cohorts report possible associations between maternal mobile phone use during pregnancy and later behavioral problems or attention difficulties, but the effect sizes are modest and susceptible to recall bias, residual confounding, and exposure misclassification.[142,143] Systematic reviews in children and adolescents note heterogeneity in methods and inconsistent findings for neurocognitive outcomes, with no clear dose-response across modern typical exposures.[4,12,28] Proposed mechanisms include thermal effects at high specific absorption rates and non-thermal pathways such as oxidative stress or altered calcium signaling, yet these remain speculative at environmental exposure levels. In practice, proportionate mitigation is reasonable. Families can use speaker mode or wired headsets, avoid carrying active phones directly against the abdomen for long periods, and limit prolonged calls, while clinicians should emphasize that current evidence does not support a strong cause.

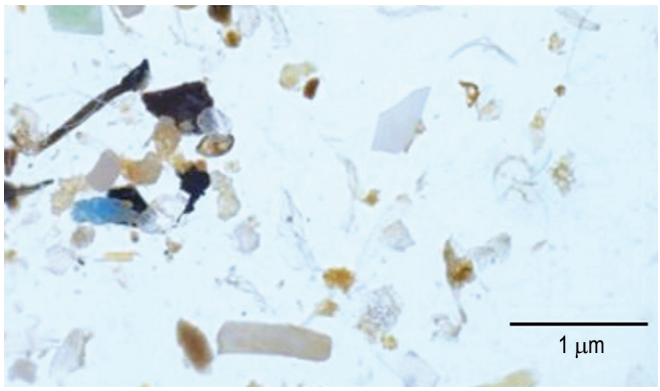


Fig. 6: Microplastics may affect infant health because they are going through sensitive stages of growth and development; studies have detected microplastics in the placenta, breast milk, and infant stool. Laboratory and animal studies suggest that these can cause inflammation, oxidative stress, disrupt gut microbiota, and may carry harmful endocrine-disrupting additives

Chemical Exposures

Flame retardants such as polybrominated diphenyl ethers (PBDEs) can bioaccumulate and cross the placenta.[144] Prenatal exposure to these chemicals has been associated with lower developmental quotients and attention problems in young children, with thyroid hormone disruption as a plausible mechanism because thyroid hormone is critical for neuronal migration and myelination.[6,8,145,146] Practical mitigation includes advising on cleaner air strategies during pregnancy, avoiding tobacco smoke and high traffic outdoor activity on poor air quality days, choosing low mercury fish while maintaining omega-3 intake, using safe water sources in arsenic endemic regions, and reducing contact with plastics and dust reservoirs that contain PBDEs through simple household practices.[147] Communication should emphasize proportionate action. Most single exposures confer small absolute risks, and supportive prenatal care alongside targeted exposure reduction can help shift neurodevelopmental trajectories toward more favorable outcomes.[148]

Organophosphate pesticides remain the most consistently linked chemical class for prenatal neurotoxicity.[149] Prospective cohort studies that measured maternal urinary dialkyl phosphate metabolites report dose-related developmental delay and attention deficits, with effect sizes in the low-moderate range and strongest signals when exposure spans mid to late gestation.[6,145,146] Findings from urban cohorts that quantified chlorpyrifos using umbilical cord blood or personal air monitoring add convergent evidence, including developmental delay, working memory deficits, and structural brain differences in exposed children.[150,151] Mechanistically, organophosphates inhibit acetylcholinesterase and can impair axonal growth, neurite outgrowth, and synaptogenesis at doses that only partially inhibit the enzyme.[152] Additional pathways include oxidative stress, thyroid disruption, and altered microglial activation, which can perturb cortical layering and fronto-striatal network development.[153]

Household chemicals and solvents show a more heterogeneous exposure profile.[154] Maternal exposure to common glycol ethers,

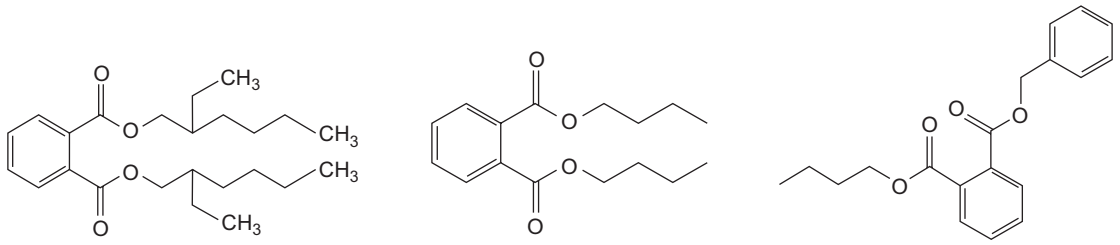


Fig. 7: Phthalates are organic compounds that are structurally defined as diesters of phthalic acid. The core structure consists of a benzene ring with two ester functional groups (–COO–) attached to adjacent carbon atoms in the ortho (1,2-) position. Each ester group is formed by the reaction of phthalic acid with an alcohol, resulting in two alkyl or aryl side chains (commonly denoted as R) extending from the ring. The length and branching of these R groups vary in different phthalates

ketones, and other solvents in occupational or household contexts is associated with small deficits in psychomotor function and attention during infancy and toddlerhood, with dose and chronicity shaping effect size.[155] For most consumer uses, exposure levels are lower in newer than in historical occupational settings, and risk can be reduced through simple ventilation, limiting use of aerosolized products, and substituting less-volatile formulations.[156] All avoidable pesticides should be avoided during pregnancy; if needed, these should be stored outdoors and replaced by mechanical/bait-based controls.[157] Solvents should be used for the shortest times possible in well-ventilated areas, with adherence to label precautions.[158]

Maternal Lifestyle

Lifestyle exposures in pregnancy tend to converge on a small set of biological pathways that matter for the fetal brain: Oxidative and inflammatory tone, endocrine and HPA signaling, placental perfusion and transport, and the adequacy and timing of key nutrients.[159] Alcohol is the clearest example. It crosses the placenta, perturbs neurogenesis and synaptogenesis, and produces dose-related structural and functional effects that translate into attention, learning, and behavioral differences.[160] Complete avoidance remains the only reliably safe advice.[8]

- Tobacco smoke and vaping sustain hypoxia, oxidative stress, and endothelial dysfunction at the maternal–placental interface; nicotine also alters cholinergic signaling that supports circuit assembly.[161] Substituting e-cigarettes lowers combustion products but adds solvents and particulates, so cessation is still the clinical goal.[8] Caffeine, while less potent, crosses the placenta and is cleared slowly in pregnancy. Higher intakes track with lower birth weight and small behavioral shifts in observational cohorts, so a pragmatic ceiling near 200 mg per day, counting all sources, is reasonable.[8]
- Medication and drug exposures should be framed by risk–benefit, not fear. For epilepsy, valproate carries a clear neurodevelopmental risk that supports use only when alternatives are not viable, with folate optimization and shared decision-making.[32] By contrast, associations between selective serotonin reuptake inhibitors and autism are small and likely confounded by indication; undertreating maternal depression or anxiety prolongs stress biology and sleep loss that are themselves adverse for fetal development.[162] Management should prioritize maternal mental health stability using psychological therapies and, when indicated, pharmacotherapy at the lowest effective dose in coordination with obstetrics and psychiatry.[33,121]
- Sleep and nutrition knit these themes together. Sleep deprivation and circadian disruption magnify insulin resistance, inflammation, and cortisol exposure, which can erode placental barrier function and shift fetal stress signaling.[163] Hyperemesis and restrictive diets introduce energy and micronutrient shortages that intersect with thyroid function and one-carbon metabolism; timely treatment, weight restoration, and attention to iodine, iron, folate, and vitamin D sufficiency are practical levers, especially where baseline intake is marginal.[24,121]
- Physical activity, within obstetric guidance, improves glycemic control, gestational weight gain, mood, and sleep, thereby reducing the same inflammatory and endocrine loads that propagate risk.[164] The clinical message is continuity rather than fragments: support cessation of alcohol and nicotine, keep caffeine modest, individualize essential medicines, protect sleep, correct malnutrition early, and promote regular moderate movement.[165] These coordinated adjustments lower cumulative biological strain on the placenta and provide a realistic path to better neurodevelopmental trajectories.[121]

Maternal Obstetric and Perinatal Factors

- Hypertensive disorders of pregnancy, including gestational hypertension and pre-eclampsia, signal placental vascular stress that can recalibrate fetal brain development through intermittent hypoxia, oxidative stress, and altered endocrine transport across the placenta.[166] Large population studies and meta-analyses show small but consistent increases in the odds of autism-spectrum conditions, ADHD, and sometimes intellectual disability among exposed offspring, with the highest risks seen in early onset or severe pre-eclampsia, where placental malperfusion and inflammatory signaling are greater.[18,167] These data support close blood pressure control, aspirin prophylaxis when indicated, and vigilant surveillance of fetal growth and perfusion as measures that improve obstetric safety and likely optimize the neurodevelopmental context.[168]
- Preterm birth concentrates risk because it shortens *in utero* brain growth and exposes an immature brain to extrauterine stressors during rapid white matter development.[169] Very preterm birth is associated with higher rates of attentional difficulties and ADHD traits, with variability driven by neonatal complications, severity of illness, and social context.[170] Intrauterine growth restriction (IUGR) and small for gestational age (SGA) status add an independent burden. Placental insufficiency with brain-sparing redistribution may preserve head size yet still alter cortical and thalamocortical maturation.[171] Meta-analytic evidence shows lower mean cognitive scores in children with antenatal IUGR or SGA at both term and preterm gestations, suggesting additive vascular, metabolic, and inflammatory pathways that shape synaptogenesis and myelination.[172]
- Acute perinatal complications operate through different mechanisms. Sentinel hypoxic ischemic events can produce basal ganglia, thalamic, or watershed white matter injury with later motor and cognitive sequelae despite modern neonatal care.[173] Therapeutic hypothermia improves outcomes but does not fully eliminate risk, which underscores the importance of prevention and timely intrapartum management.[174]
- Mode of delivery appears relevant mainly through effects on early microbial colonization rather than direct mechanical effects.[175] Infants born by cesarean section acquire distinct initial microbiota profiles compared with those delivered vaginally, with differences in *Bacteroides* and other taxa that can influence immune training in early life.[176] Long-term neurodevelopmental differences by delivery mode are small after adjustment for indication and maternal factors, so counseling should focus on evidence-based obstetric indications for cesarean delivery and on postnatal practices that support healthy microbial seeding, including skin-to-skin care and breastfeeding.[177]

Taken together, these obstetric and perinatal exposures converge on placental perfusion, inflammatory tone, timing of nutrient and hormone delivery, and the early maturation of brain and immune systems.[178,179] A practical stance is to prevent and treat hypertensive disease, monitor fetal growth, minimize iatrogenic prematurity, optimize intrapartum safety, and strengthen postnatal environments that promote recovery and resilience.[180,181]

Social and Psychosocial Factors

- Social context shapes biology during pregnancy in ways that matter for the developing brain.[182] Domestic violence, poverty, low maternal education, limited social support, and heavy unpaid or paid workload raise chronic stress biology and erode conditions for healthy sleep, nutrition, and prenatal care.[183,184] These pressures increase maternal anxiety and depressive symptoms, elevate cortisol and inflammatory tone, and strain placental function, which together can influence fetal neuronal migration, synaptogenesis, and stress system calibration.[13,185]
- Maternal influences on early neurodevelopment rarely act in isolation. Exposures cluster across time and context, and their biological effects converge on shared pathways that regulate placentation, immune tone, oxidative balance, and neurodevelopmental patterning.[186,187] Empirically, combined burdens tend to produce larger effects than single exposures, consistent with models that treat pregnancy as a system-level stress test of neurodevelopmental plasticity.[14,188]
- Domestic violence during pregnancy is associated with maternal depression, inadequate weight gain, and obstetric complications, and with higher risks of low birth weight and preterm birth, which are established pathways to later attentional and learning difficulties.[170,189] Poverty and education gradients add cumulative exposure.[190] Lower family income and parental education correlate with differences in cortical surface area and white matter microstructure in childhood, which likely reflect the combined effects of stress, reduced enrichment, and constrained access to healthful environments and care.[191,192] Nutrition insecurity compounds risk by limiting intake of iodine, iron, and other nutrients that support myelination and neurotransmitter synthesis and is linked to suboptimal gestational weight gain and altered infant cognitive and language outcomes.[20,24,27]
- Lack of dependable social support removes a natural buffer against stress. Support from partners, family, and peers is linked to lower perceived stress, fewer depressive symptoms, and better obstetric outcomes, plausibly through flatter cortisol profiles and reduced inflammation.[5,121] Maternal workload adds another layer. Physically demanding or long working hours are associated with higher risks of preterm birth and fetal growth restriction in observational studies, especially when combined with prolonged standing or shift work, which implies more frequent hypoxia and inflammatory signaling at the maternal–placental interface.[23,92,172]

  Psychosocial stress frequently amplifies the impact of physical pollutants.[193] Stress primes inflammatory and adrenergic pathways, lowering the threshold for injury from airborne toxicants.[194] In human cohorts, maternal stress and prenatal exposure to traffic-related pollution often led to greater behavioral problems and lower neurocognitive performance than peers exposed to either factor alone.[188,195] There are plausible mechanisms; stress hormones and cytokines increase placental permeability and microglial reactivity, while fine particles and nitrogen dioxide drive oxidative stress; the combined signal intensifies IL-6 and TNF cascades that shape neuronal migration and synaptic pruning during critical windows.[188,195]
- Diet and metabolic status also condition inflammatory tone, creating fertile ground for interaction with other risks.[196] In overweight/obese pregnant women, low-grade inflammation is seen frequently and can be attenuated by higher omega-3 intake, which reduces placental and adipose expression of inflammatory mediators (as described above).[89,105,197]

For clinicians, these findings argue for routine inquiry about the safety of pregnant women at home, food access, social support, education barriers, and workload.[198] Brief interventions have leverage. Safety planning and linkage to domestic violence services, cash or in-kind nutrition support, peer or partner-based support programs, and employer accommodations to reduce heavy physical demands can lower cumulative stress loads.[199,200] Clear messaging helps families engage without stigma. Most single risks carry small absolute effects, but reducing more than one can shift the biochemical setting in which the fetal brain is built and improve the odds of healthier attention, emotional regulation, language, and behavior trajectories.[121]

Mechanistic Pathways Linking Maternal Factors and Neurodevelopment

- Gene–environment interplay refines vulnerability.[201] Variants in neurodevelopmental or detoxification pathways can modify risk from specific exposures. One example is the MET proto-oncogene, receptor tyrosine kinase; variant rs1858830.[35] Children with the MET CC genotype who also experienced higher prenatal exposure to traffic-related air pollution had increased odds of autism compared with children without that combined profile, suggesting multiplicative risk rather than simple addition.[35] Similarly, one-carbon metabolism genotypes in mothers or infants modify the association between periconceptional prenatal vitamin use and autism risk, indicating that nutritional supports can buffer genetic susceptibility when delivered at the right time.[34,115] These findings align with the exposome framework, which treats early-life health as the product of many correlated exposures interacting with the genome across time and tissues.[14]
- Epigenetic programming is important around conception and early implantation.[64] Maternal nutrition, endocrine milieu, and inflammation shape DNA methylation and histone marks in placenta and fetal brain, with durable effects on gene networks that govern neurogenesis, axon guidance, and synaptogenesis.[47] These marks integrate with fetal genotype to modulate susceptibility to seemingly-similar exposures.[202]
- Disruption of neurotransmitter systems is another common thread. Maternal stress and inflammatory cytokines modify placental tryptophan transport and kynurenine pathway flux, reducing serotonin availability in the fetal brain.[203] In parallel, altered glucocorticoid signaling and cytokine tone change dopaminergic and GABAergic maturation in fronto-striatal and limbic circuits that regulate attention and emotion.[204] These changes offer a plausible bridge to later ADHD symptoms and emotional dysregulation when exposures occur during mid to late gestation.[21,111]
- Altered neuronal migration and synaptic pruning tie the pathways together.[205] Cytokine and oxidative environments influence radial migration and interneuron positioning, while microglia and complement pathways govern activity-dependent elimination of synapses.[206] Human genetic studies implicate complement component 4 variation in schizophrenia risk.[123] Some experimental studies show that microglia-dependent pruning is sensitive to immune cues; altered pruning can lead to atypical network refinement.[207] These lines of evidence support a model in which maternal inflammatory tone and oxidative

stress recalibrate the timing and amount of pruning during sensitive windows.[44,208]

Together, these mechanisms show how diverse maternal states converge on a limited set of biological switches that set developmental trajectories. The same switches are amenable to prevention through improved metabolic health, infection control, balanced nutrition, mental health treatment, and reduction of avoidable toxicants.

Protective Factors

- Protective care in pregnancy works because many risks converge on a few modifiable pathways.[209] Good prenatal care identifies hypertension, diabetes, thyroid dysfunction, infection, malnutrition, and intimate partner violence early, then treats or buffers them before they translate into sustained placental inflammation or endocrine disruption.[210–212] This is the simplest route to reduce cumulative cytokine load, improve nutrient timing, and stabilize fetal growth signals.[25,121]
- Micronutrient sufficiency is foundational. Periconceptional folic acid prevents neural tube defects and is associated with lower autism risk in several cohorts, which aligns with one-carbon metabolism and methylation biology.[34,213] Iodine sufficiency supports fetal thyroid-dependent neurogenesis and myelination.[86] Attention to iron, vitamin D, and overall diet quality helps maintain myelination, neurotransmitter synthesis, and placental immune balance, particularly in settings with marginal baseline intake.[24]
- Maternal mental health support can increase biological resilience.[214,215] Treating antenatal depression and anxiety with evidence-based psychological therapies and, when indicated, pharmacotherapy lowers chronic cortisol exposure and improves sleep, appetite, and engagement with prenatal care.[5] These changes are linked to better behavioral and language outcomes in children, with the greatest benefit when symptoms remit during pregnancy or early postpartum.[13,110]
- Dietary patterns can shift inflammatory tone.[10,17,21,28,89,104,110,111] Practical steps include steady protein and fiber, abundant vegetables and fruits, iodine and iron adequacy, and marine omega-3 long-chain fatty acids when acceptable.[216] In women with obesity, omega-3 supplementation reduces placental and systemic inflammatory markers, which offers a plausible pathway to support neurodevelopmental resilience.[216,217]
- Social support is a biological intervention as much as a social one.[198] Reliable partner, family, and peer support are associated with flatter diurnal cortisol, fewer depressive symptoms, better obstetric outcomes, and improved early caregiving.[218,219] Screening for food insecurity and unsafe housing, then linking families to assistance, reduces background stress and helps close nutrition gaps that affect myelination and neurotransmitter pathways.[3,19,20,24,27,47,115]
- Mindfulness and related stress reduction programs are feasible during pregnancy and can reduce perceived stress, anxiety, and depressive symptoms while improving sleep.[220,221] These effects plausibly operate through lower HPA activation and improved autonomic balance during sensitive windows.[4,5,12,19,37,92,110,125,188,222]
- Routine physical activity within obstetric guidance improves glycemic control, gestational weight gain, and sleep, and it lowers systemic inflammation.[223,224] Framed for safety and access, regular moderate movement complements nutrition and mental health support to keep placental signaling in a healthier range.[121,224]
- Reduction in exposure to avoidable toxicants could trim the cumulative burden of disease.[225] Practical steps include smoke-free homes, cleaner indoor air on poor outdoor air quality days, improved nutrition, while limiting mercury, safe water sources in arsenic endemic regions, and lower contact with plastics and dust that contain endocrine disruptors or flame retardants.[226–232] These are small adjustments that add up, especially when combined with the supports above.[8,131,138,139,145,146]

Clinical Evaluation

Maternal Evaluation for Neurodevelopmental Risk

A developmental visit is an opportunity to map the intrauterine ecology that shaped the child.[214] The clinician should ask about

- Preconception and antenatal health.
- Screen for hypertensive disorders, gestational diabetes, thyroid disease, autoimmune conditions, clinically significant infections or fever, and severe hyperemesis.
- Review medications and substances, including antiepileptics (valproate), antidepressants, alcohol, tobacco, vaping, caffeine, and illicit drugs.
- Elicit sleep quality, weight gain pattern, diet quality, and access to food, iodine and folate use, and physical activity.
- Ask about environmental exposures that are common and modifiable, such as high traffic air pollution at home or work, pesticide use, solvent use in poorly ventilated spaces, and use of plastics with food.
- Psychosocial factors: Domestic violence, depressive or anxiety symptoms, trauma, social support, workload, housing stability, and financial strain.

All these domains link to placental inflammation, endocrine timing, nutrient delivery, or fetal stress signaling, the same pathways that recur across this review.[8,25,121]

Maternal Counseling

Counseling converts probabilistic science into everyday habits that lower cumulative biological strain on the placenta and fetal brain.[233,234] Practical messages have the most value: To encourage complete avoidance of alcohol and tobacco, modest caffeine intake, and safe, necessary use of medicines with shared decision-making for psychotropics and antiepileptics; reinforce periconceptional and antenatal folic acid, iodine sufficiency, and attention to iron and vitamin D in settings with marginal intake; normalize sleep care, brief stress reduction practices, and regular moderate activity as treatments that improve glycemic control, lower inflammation, and steady cortisol exposure.[235,236] We offer simple exposure reduction steps that do not create fear: cleaner indoor air on poor air quality days, species-specific fish choices that preserve omega-3 intake while limiting mercury, ventilation with household solvents, and integrated pest management rather than frequent organophosphate use.[34,86,89,121,145]

Preventive Strategies in Public Health

Population risk shifts when supports are routine rather than exceptional.[237] Four levers stand out: (1) Universal or targeted fortification and supplementation programs for folate and iodine reduce neural tube defects and support thyroid-dependent myelination; (2) systematic screening and stepped care for perinatal

depression and anxiety improve maternal function and may reduce child behavioral risk by lowering chronic cortisol exposure and improving caregiving; (3) environmental policy matters: tobacco control, clean air standards, arsenic safe water, and reduced use of high risk pesticides lower background toxic load for entire communities; and (4) social protection works as biology.[8,86,121,145,213] Cash or in-kind supports that reduce food insecurity and housing instability, paid leave, and workplace accommodations that limit heavy physical demands and remove night shift work in pregnancy can reduce stress and improve obstetric outcomes that are upstream of attention, language, and behavior.[5,191] Developmental pediatricians can help optimize care by documenting needs, partnering with obstetrics and public health, and embedding warm handoffs to nutrition, mental health, social work, and early intervention.[238]

Gaps in Current Research

Much of what we know about maternal influences on neurodevelopment comes from high-income settings, which limits how confidently we can generalize to regions where exposure profiles, infection burdens, dietary patterns, and access to prenatal care differ.[239–242] Low- and middle-income countries remain underrepresented, and this skews estimates for common realities such as biomass fuel exposure, groundwater contaminants, seasonal food insecurity, informal work, and constrained health services.[241–243] In most developing countries, there are few large prospective birth cohorts with repeated antenatal sampling, detailed obstetric phenotyping, and standardized neurodevelopmental assessments that extend into school years.[244,245] Without such long-horizon designs, timing effects are blurred, prenatal and postnatal influences are hard to separate, and the impact of state programs for nutrition, pollution control, and primary care cannot be evaluated with precision.[246] An additional limitation is that most studies have focused on single hazards in isolation.[247] We have limited data on how exposures combine, sequence, and interact across stages of pregnancy and, consequently, lack adequate analytic frameworks.[248]

A second frontier concerns the reversibility of problems.[249] Epigenetic changes in the placenta and fetal tissues can reflect maternal nutrition, endocrine status, and inflammation, although we cannot always differentiate between transient, durable, and reversible marks yet.[250,251] Trials that pair biologic markers with clinical outcomes are needed to map intervention windows and to confirm that moving a biomarker is actually clinically effective.[252] We need pragmatic risk stratification that combines maternal history, simple biomarkers, and context to direct the right support to the right patients.[253] Personalized antenatal packages could prioritize depression care and sleep for one patient, iodine and iron sufficiency for another, or tighter blood pressure and glucose control for a third, all while addressing local toxicants and social stressors.[254] Implementation strategies are needed to direct tailored packages in routine antenatal care, which measure uptake and equity, and track outcomes.[255]

Conclusion

The maternal environment during the preconception/antenatal periods is a key determinant of how well the fetal neural circuits emerge and refine.[256,257] This influence is not linear; multiple signals accumulate and interact.[257] These efforts need to be continued with the same intensity for both the mother and the infant after birth.[258] Metabolic status, immune tone, endocrine balance, nutrient availability, toxicant exposure, sleep, stress, and social conditions converge on a small number of neurobiological pathways that govern nutrient timing, hormone signaling, oxidative balance, microglial priming, neurotransmitter availability, neuronal migration, and synaptic pruning.[45,259] Different combinations can result in similar phenotypes, and the same exposure can result in different outcomes because vulnerability is conditioned by genetics, timing, and context.[260] We need to develop timely, interdisciplinary, effective, and sustainable interventions.[261]

Orcid

Akhil Maheshwari https://orcid.org/0000-0003-3613-4054

References

1. Happe F, Frith U. Annual research review: Looking back to look forward – Changes in the concept of autism and implications for future research. J Child Psychol Psychiatry 2020;61(3):218–232. DOI: 10.1111/jcpp.13176.
2. Banik A, Kandilya D, Ramya S, et al. Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. Genes (Basel) 2017;8(6):150. DOI: 10.3390/genes8060150.
3. Cryan JF, O'Riordan KJ, Cowan CSM, et al. The microbiota-gut-brain axis. Physiol Rev 2019;99(4):1877–2013. DOI: 10.1152/physrev.00018.2018.
4. De Domenico C, Calderone A, Latella D, et al. Prenatal psychological distress and neurodevelopmental trajectories in the first 3 years: A systematic review. BMJ Open 2025;15(11):e104716. DOI: 10.1136/bmjopen-2025-104716.
5. Schetter CD, Tanner L. Anxiety, depression and stress in pregnancy: Implications for mothers, children, research, and practice. Curr Opin Psychiatry 2012;25(2):141–148. DOI: 10.1097/YCO.0b013e3283503680.
6. Eskenazi B, Marks AR, Bradman A, et al. Organophosphate pesticide exposure and neurodevelopment in young Mexican-American children. Environ Health Perspect 2007;115(5):792–798. DOI: 10.1289/ehp.9828.
7. Garcia-Serna AM, Morales E. Neurodevelopmental effects of prenatal vitamin D in humans: Systematic review and meta-analysis. Mol Psychiatry 2020;25(10):2468–2481. DOI: 10.1038/s41380-019-0357-9.
8. Grandjean P, Landrigan PJ. Neurobehavioural effects of developmental toxicity. Lancet Neurol 2014;13(3):330–338. DOI: 10.1016/S1474-4422(13)70278-3.
9. Guan J, Qiu J, Li L, et al. A meta-analysis of adverse offspring health outcomes in patients with gestational diabetes mellitus. Diabetes Obes Metab 2025;27(7):3555–3567. DOI: 10.1111/dom.16341.
10. Han VX, Patel S, Jones HF, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: A systematic review. Transl Psychiatry 2021;11(1):71. DOI: 10.1038/s41398-021-01198-w.
11. Insel TR. Rethinking schizophrenia. Nature 2010;468(7321):187–193. DOI: 10.1038/nature09552.
12. Manzari N, Matvienko-Sikar K, Baldoni F, et al. Prenatal maternal stress and risk of neurodevelopmental disorders in the offspring: A systematic review and meta-analysis. Soc Psychiatry Psychiatr Epidemiol 2019;54(11):1299–1309. DOI: 10.1007/s00127-019-01745-3.
13. O'Donnell KJ, Meaney MJ. Fetal origins of mental health: The developmental origins of health and disease hypothesis. Am J Psychiatry 2017;174(4):319–328. DOI: 10.1176/appi.ajp.2016.16020138.
14. Vrijheid M, Slama R, Robinson O, et al. The human early-life exposome (HELIX): Project rationale and design. Environ Health Perspect 2014;122(6):535–544. DOI: 10.1289/ehp.1307204.
15. Zhang S, Lin T, Zhang Y, et al. Effects of parental overweight and obesity on offspring's mental health: A meta-analysis of observational studies. PLoS One 2022;17(12):e0276469. DOI: 10.1371/journal.pone.0276469.

16. Biagioli V, Matera M, Ramenghi LA, et al. Microbiome and pregnancy dysbiosis: A narrative review on offspring health. Nutrients 2025;17(6):1033. DOI: 10.3390/nu17061033.
17. Bilbo SD, Block CL, Bolton JL, et al. Beyond infection – Maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. Exp Neurol 2018;299(Pt A):241–251. DOI: 10.1016/j.expneurol.2017.07.002.
18. Brand JS, Lawlor DA, Larsson H, et al. Association between hypertensive disorders of pregnancy and neurodevelopmental outcomes among offspring. JAMA Pediatr 2021;175(6):577–585. DOI: 10.1001/jamapediatrics.2020.6856.
19. Glover V, O'Donnell KJ, O'Connor TG, et al. Prenatal maternal stress, fetal programming, and mechanisms underlying later psychopathology: A global perspective. Dev Psychopathol 2018;30(3):843–854. DOI: 10.1017/S095457941800038X.
20. Prado EL, Dewey KG. Nutrition and brain development in early life. Nutr Rev 2014;72(4):267–284. DOI: 10.1111/nure.12102.
21. Sullivan EL, Riper KM, Lockard R, et al. Maternal high-fat diet programming of the neuroendocrine system and behavior. Horm Behav 2015;76:153–161. DOI: 10.1016/j.yhbeh.2015.04.008.
22. Van den Bergh BRH, van den Heuvel MI, Lahti M, et al. Prenatal developmental origins of behavior and mental health: The influence of maternal stress in pregnancy. Neurosci Biobehav Rev 2020;117:26–64. DOI: 10.1016/j.neubiorev.2017.07.003.
23. Wang H, Laszlo KD, Gissler M, et al. Maternal hypertensive disorders and neurodevelopmental disorders in offspring: A population-based cohort in two Nordic countries. Eur J Epidemiol 2021;36(5):519–530. DOI: 10.1007/s10654-021-00756-2.
24. Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382(9890):427–451. DOI: 10.1016/S0140-6736(13)60937-X.
25. Burton GJ, Fowden AL, Thornburg KL. Placental origins of chronic disease. Physiol Rev 2016;96(4):1509–1565. DOI: 10.1152/physrev.00029.2015.
26. Carter SA, Lin JC, Chow T, et al. Maternal obesity, diabetes, preeclampsia, and asthma during pregnancy and likelihood of autism spectrum disorder with gastrointestinal disturbances in offspring. Autism 2023;27(4):916–926. DOI: 10.1177/13623613221118430.
27. Laraia BA, Siega-Riz AM, Gundersen C. Household food insecurity is associated with self-reported pregravid weight status, gestational weight gain, and pregnancy complications. J Am Diet Assoc 2010;110(5):692–701. DOI: 10.1016/j.jada.2010.02.014.
28. Modabbernia A, Velthorst E, Reichenberg A. Environmental risk factors for autism: An evidence-based review of systematic reviews and meta-analyses. Mol Autism 2017;8:13. DOI: 10.1186/s13229-017-0121-4.
29. Xu G, Jing J, Bowers K, et al. Maternal diabetes and the risk of autism spectrum disorders in the offspring: A systematic review and meta-analysis. J Autism Dev Disord 2014;44(4):766–775. DOI: 10.1007/s10803-013-1928-2.
30. Atladottir HO, Thorsen P, Ostergaard L, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. J Autism Dev Disord 2010;40(12):1423–1430. DOI: 10.1007/s10803-010-1006-y.
31. Chen SW, Zhong XS, Jiang LN, et al. Maternal autoimmune diseases and the risk of autism spectrum disorders in offspring: A systematic review and meta-analysis. Behav Brain Res 2016;296:61–69. DOI: 10.1016/j.bbr.2015.08.035.
32. Christensen J, Gronborg TK, Sorensen MJ, et al. Prenatal valproate exposure and risk of autism spectrum disorders and childhood autism. JAMA 2013;309(16):1696–1703. DOI: 10.1001/jama.2013.2270.
33. Rai D, Lee BK, Dalman C, et al. Parental depression, maternal antidepressant use during pregnancy, and risk of autism spectrum disorders: Population based case-control study. BMJ 2013;346:f2059. DOI: 10.1136/bmj.f2059.
34. Schmidt RJ, Tancredi DJ, Ozonoff S, et al. Maternal periconceptional folic acid intake and risk of autism spectrum disorders and developmental delay in the CHARGE (CHildhood Autism Risks from Genetics and Environment) case-control study. Am J Clin Nutr 2012;96(1):80–89. DOI: 10.3945/ajcn.110.004416.
35. Volk HE, Kerin T, Lurmann F, et al. Autism spectrum disorder: Interaction of air pollution with the MET receptor tyrosine kinase gene. Epidemiology 2014;25(1):44–47. DOI: 10.1097/EDE.0000000000000030.
36. Zwaigenbaum L, Bauman ML, Choueiri R, et al. Early identification and interventions for autism spectrum disorder: Executive summary. Pediatrics 2015;136(Suppl 1):S1–S9. DOI: 10.1542/peds.2014-3667B.
37. Garner AS, Shonkoff JP, Siegel BS, et al. Early childhood adversity, toxic stress, and the role of the pediatrician: Translating developmental science into lifelong health. Pediatrics 2012;129(1):e224–e231. DOI: 10.1542/peds.2011-2662.
38. Uddin LQ, Dajani DR, Voorhies W, et al. Progress and roadblocks in the search for brain-based biomarkers of autism and attention-deficit/hyperactivity disorder. Transl Psychiatry 2017;7(8):e1218. DOI: 10.1038/tp.2017.164.
39. Mohammad SI, Azzam ER, Vasudevan A, et al. Precision neurodiversity: Personalized brain network architecture as a window into cognitive variability. Front Hum Neurosci 2025;19:1669431. DOI: 10.3389/fnhum.2025.1669431.
40. Beck KB, Ionadi A, Wagner T, et al. The schools unified in neurodiversity collaborative: Co-designing a program to enhance educator knowledge and efficacy supporting children with neurodevelopmental disabilities. Autism 2025;13623613251388627. DOI: 10.1177/13623613251388627.
41. Stenning A, Bertilsdotter-Rosqvist H. Neurodiversity studies: Mapping out possibilities of a new critical paradigm. Disabil Soc 2021;36(9):1532–1537. DOI: 10.1080/09687599.2021.1919503.
42. Sharpee TO, Destexhe A, Kawato M, et al. 25th Annual Computational Neuroscience Meeting: CNS-2016. BMC Neurosci 2016;17(Suppl 1):54. DOI: 10.1186/s12868-016-0283-6.
43. Delmonte S, Gallagher L, O'Hanlon E, et al. Functional and structural connectivity of frontostriatal circuitry in Autism Spectrum Disorder. Front Hum Neurosci 2013;7:430. DOI: 10.3389/fnhum.2013.00430.
44. Stiles J, Jernigan TL. The basics of brain development. Neuropsychol Rev 2010;20(4):327–348. DOI: 10.1007/s11065-010-9148-4.
45. Lacagnina S. The Developmental origins of health and disease (DOHaD). Am J Lifestyle Med 2020;14(1):47–50. DOI: 10.1177/1559827619879694.
46. Barker DJ. The origins of the developmental origins theory. J Intern Med 2007;261(5):412–417. DOI: 10.1111/j.1365-2796.2007.01809.x.
47. Fleming TP, Watkins AJ, Velazquez MA, et al. Origins of lifetime health around the time of conception: Causes and consequences. Lancet 2018;391(10132):1842–1852. DOI: 10.1016/S0140-6736(18)30312-X.
48. Jakovcevski M, Akbarian S. Epigenetic mechanisms in neurological disease. Nat Med 2012;18(8):1194–1204. DOI: 10.1038/nm.2828.
49. Yao B, Christian KM, He C, et al. Epigenetic mechanisms in neurogenesis. Nat Rev Neurosci 2016;17(9):537–549. DOI: 10.1038/nrn.2016.70.
50. Albert M, Huttner WB. Epigenetic and transcriptional pre-patterning – An emerging theme in cortical neurogenesis. Front Neurosci 2018;12:359. DOI: 10.3389/fnins.2018.00359.
51. Raikkonen K, Pesonen AK, Roseboom TJ, et al. Early determinants of mental health. Best Pract Res Clin Endocrinol Metab 2012;26(5):599–611. DOI: 10.1016/j.beem.2012.03.001.
52. Kostovic I, Judas M. The development of the subplate and thalamocortical connections in the human foetal brain. Acta Paediatr 2010;99(8):1119–1127. DOI: 10.1111/j.1651-2227.2010.01811.x.
53. Bale TL. The placenta and neurodevelopment: Sex differences in prenatal vulnerability. Dialogues Clin Neurosci 2016;18(4):459–464. DOI: 10.31887/DCNS.2016.18.4/tbale.
54. Bale TL. Epigenetic and transgenerational reprogramming of brain development. Nat Rev Neurosci 2015;16(6):332–344. DOI: 10.1038/nrn3818.
55. Curley JP, Jensen CL, Mashoodh R, et al. Social influences on neurobiology and behavior: Epigenetic effects during development.

Psychoneuroendocrinology 2011;36(3):352–371. DOI: 10.1016/j.psyneuen.2010.06.005.
56. Hodes GE. Sex, stress, and epigenetics: Regulation of behavior in animal models of mood disorders. Biol Sex Differ 2013;4(1):1. DOI: 10.1186/2042-6410-4-1.
57. Thorsheim C, Khan S, Lu Y, et al. Maternal exacerbating and protective factors that shape the prevalence and severity of child attention-deficit hyperactivity disorder: A narrative review. Front Psychiatry 2025;16:1577707. DOI: 10.3389/fpsyt.2025.1577707.
58. Demontis D, Duan J, Hsu YH, et al. Rare genetic variants confer a high risk of ADHD and implicate neuronal biology. Nature 2025. DOI: 10.1038/s41586-025-09702-8.
59. Chen CC, Lin CH, Lin MC. Maternal autoimmune disease and risk of offspring autism spectrum disorder – A nationwide population-based cohort study. Front Psychiatry 2023;14:1254453. DOI: 10.3389/fpsyt.2023.1254453.
60. Dumolt JH, Powell TL, Jansson T. Placental function and the development of fetal overgrowth and fetal growth restriction. Obstet Gynecol Clin North Am 2021;48(2):247–266. DOI: 10.1016/j.ogc.2021.02.001.
61. Bhattacharya A, Freedman AN, Avula V, et al. Placental genomics mediates genetic associations with complex health traits and disease. Nat Commun 2022;13(1):706. DOI: 10.1038/s41467-022-28365-x.
62. Ursini G, Punzi G, Langworthy BW, et al. Placental genomic risk scores and early neurodevelopmental outcomes. Proc Natl Acad Sci U S A 2021;118(7):e2019789118. DOI: 10.1073/pnas.2019789118.
63. Ge F, Wang Y, Liu X, et al. Association between maternal genome-wide polygenic scores for psychiatric and neurodevelopmental disorders and adverse perinatal events: A Danish population-based study. Biol Psychiatry Glob Open Sci 2026;6(1):100613. DOI: 10.1016/j.bpsgos.2025.100613.
64. Zuccarello D, Sorrentino U, Brasson V, et al. Epigenetics of pregnancy: Looking beyond the DNA code. J Assist Reprod Genet 2022;39(4):801–816. DOI: 10.1007/s10815-022-02451-x.
65. Vukic M, Wu H, Daxinger L. Making headway towards understanding how epigenetic mechanisms contribute to early-life effects. Philos Trans R Soc Lond B Biol Sci 2019;374(1770):20180126. DOI: 10.1098/rstb.2018.0126.
66. Haberg SE, Page CM, Lee Y, et al. DNA methylation in newborns conceived by assisted reproductive technology. Nat Commun 2022;13(1):1896. DOI: 10.1038/s41467-022-29540-w.
67. Laufer BI, Neier K, Valenzuela AE, et al. Placenta and fetal brain share a neurodevelopmental disorder DNA methylation profile in a mouse model of prenatal PCB exposure. Cell Rep 2022;38(9):110442. DOI: 10.1016/j.celrep.2022.110442.
68. Markunas CA, Wilcox AJ, Xu Z, et al. Maternal Age at Delivery Is Associated with an Epigenetic Signature in Both Newborns and Adults. PLoS One 2016;11(7):e0156361. DOI: 10.1371/journal.pone.0156361.
69. Li Piani L, Vigano P, Somigliana E. Epigenetic clocks and female fertility timeline: A new approach to an old issue? Front Cell Dev Biol 2023;11:1121231. DOI: 10.3389/fcell.2023.1121231.
70. Wong WS, Solomon BD, Bodian DL, et al. New observations on maternal age effect on germline de novo mutations. Nat Commun 2016;7:10486. DOI: 10.1038/ncomms10486.
71. Moore AM, Xu Z, Kolli RT, et al. Persistent epigenetic changes in adult daughters of older mothers. Epigenetics 2019;14(5):467–476. DOI: 10.1080/15592294.2019.1595299.
72. Falk MJ. Neurodevelopmental manifestations of mitochondrial disease. J Dev Behav Pediatr 2010;31(7):610–621. DOI: 10.1097/DBP.0b013e3181ef42c1.
73. Wang G, Yang E, Mandhan I, et al. Population-level expression variability of mitochondrial DNA-encoded genes in humans. Eur J Hum Genet 2014;22(9):1093–1099. DOI: 10.1038/ejhg.2013.293.
74. Pinto Payares DV, Spooner L, Vosters J, et al. A systematic review on the role of mitochondrial dysfunction/disorders in neurodevelopmental disorders and psychiatric/behavioral disorders. Front Psychiatry 2024;15:1389093. DOI: 10.3389/fpsyt.2024.1389093.
75. Boone PM, Buenaventura T, King JWD, et al. X-linked competition – implications for human development and disease. Nat Rev Genet 2025;26(8):571–580. DOI: 10.1038/s41576-025-00840-3.
76. Michalczyk J, Milosz A, Gesek M, et al. Prenatal diabetes and obesity: Implications for autism spectrum disorders in offspring – A comprehensive review. Med Sci Monit 2024;30:e945087. DOI: 10.12659/MSM.945087.
77. Kong L, Chen X, Gissler M, et al. Relationship of prenatal maternal obesity and diabetes to offspring neurodevelopmental and psychiatric disorders: A narrative review. Int J Obes (Lond) 2020;44(10):1981–2000. DOI: 10.1038/s41366-020-0609-4.
78. Dominguez-Castro M, Dominguez-Galicia A, Perez-Perez O, et al. Hyperglycemia affects neuronal differentiation and Nestin, FOXO1, and LMO3 mRNA expression of human Wharton's jelly mesenchymal stem cells of children from diabetic mothers. Biochem Biophys Res Commun 2022;637:300–307. DOI: 10.1016/j.bbrc.2022.11.029.
79. Yang X, Xu Y, Gao W, et al. Hyperinsulinemia-induced microglial mitochondrial dynamic and metabolic alterations lead to neuro-inflammation in vivo and in vitro. Front Neurosci 2022;16:1036872. DOI: 10.3389/fnins.2022.1036872.
80. Liu B, Liu Y, Zhai C, et al. The multifaceted roles of fatty acids and their dysregulation in obese mothers: Potential implications for infant development. Nutr Metab (Lond) 2025;22(1):141. DOI: 10.1186/s12986-025-01009-9.
81. McNamara RK, Vannest JJ, Valentine CJ. Role of perinatal long-chain omega-3 fatty acids in cortical circuit maturation: Mechanisms and implications for psychopathology. World J Psychiatry 2015;5(1):15–34. DOI: 10.5498/wjp.v5.i1.15.
82. Robles MC, Campoy C, Fernandez LG, et al. Maternal diabetes and cognitive performance in the offspring: A systematic review and meta-analysis. PLoS One 2015;10(11):e0142583. DOI: 10.1371/journal.pone.0142583.
83. Mateu-Fabregat J, Canals J, Jardi C, et al. Associations of maternal dietary carbohydrate intake, glycemic index, and glycemic load during pregnancy with offspring neurodevelopment. Eur J Pediatr 2025;184(11):721. DOI: 10.1007/s00431-025-06519-5.
84. Faustino LC, Ortiga-Carvalho TM. Thyroid hormone role on cerebellar development and maintenance: A perspective based on transgenic mouse models. Front Endocrinol (Lausanne) 2014;5:75. DOI: 10.3389/fendo.2014.00075.
85. Tavano A, Grasso R, Gagliardi C, et al. Disorders of cognitive and affective development in cerebellar malformations. Brain 2007;130(Pt 10):2646–2660. DOI: 10.1093/brain/awm201.
86. Bath SC, Steer CD, Golding J, et al. Effect of inadequate iodine status in UK pregnant women on cognitive outcomes in their children: Results from the Avon longitudinal study of parents and children (ALSPAC). Lancet 2013;382(9889):331–337. DOI: 10.1016/S0140-6736(13)60436-5.
87. Volzke H, Caron P, Dahl L, et al. Ensuring effective prevention of iodine deficiency disorders. Thyroid 2016;26(2):189–196. DOI: 10.1089/thy.2015.0543.
88. Baptiste CG, Battista MC, Trottier A, et al. Insulin and hyperandrogenism in women with polycystic ovary syndrome. J Steroid Biochem Mol Biol 2010;122(1-3):42–52. DOI: 10.1016/j.jsbmb.2009.12.010.
89. Haghiac M, Yang XH, Presley L, et al. Dietary Omega-3 Fatty Acid Supplementation Reduces Inflammation in Obese Pregnant Women: A Randomized Double-Blind Controlled Clinical Trial. PLoS One 2015;10(9):e0137309. DOI: 10.1371/journal.pone.0137309.
90. McLernon DJ, Steyerberg EW, Te Velde ER, et al. Predicting the chances of a live birth after one or more complete cycles of in vitro fertilisation: Population based study of linked cycle data from 113 873 women. BMJ 2016;355:i5735. DOI: 10.1136/bmj.i5735.
91. Tadanki D, Kaza PS, Meisinger E, et al. Comprehensive review of the impact of maternal stress on fetal development. Pediatr Discov 2025;3(3):e70004. DOI: 10.1002/pdi3.70004.
92. Lautarescu A, Craig MC, Glover V. Prenatal stress: Effects on fetal and child brain development. Int Rev Neurobiol 2020;150:17–40. DOI: 10.1016/bs.irn.2019.11.002.

93. Shimizu Y, Sakata-Haga H, Saikawa Y, et al. Influence of immune system abnormalities caused by maternal immune activation in the postnatal period. Cells 2023;12(5):741. DOI: 10.3390/cells12050741.
94. Parisi F, Milazzo R, Savasi VM, et al. Maternal low-grade chronic inflammation and intrauterine programming of health and disease. Int J Mol Sci 2021;22(4):1732. DOI: 10.3390/ijms22041732.
95. Diaz P, Powell TL, Jansson T. The role of placental nutrient sensing in maternal-fetal resource allocation. Biol Reprod 2014;91(4):82. DOI: 10.1095/biolreprod.114.121798.
96. Brown AS, Begg MD, Gravenstein S, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatry 2004;61(8):774–780. DOI: 10.1001/archpsyc.61.8.774.
97. Brown AS, Sourander A, Hinkka-Yli-Salomaki S, et al. Elevated maternal C-reactive protein and autism in a national birth cohort. Mol Psychiatry 2014;19(2):259–264. DOI: 10.1038/mp.2012.197.
98. Moog NK, Entringer S, Heim C, et al. Influence of maternal thyroid hormones during gestation on fetal brain development. Neuroscience 2017;342:68–100. DOI: 10.1016/j.neuroscience.2015.09.070.
99. Moreno-Reyes R, Fuentes Pena C, Nunez JF, et al. Critical role of iodine and thyroid hormones during pregnancy. Int J Mol Sci 2025;26(21):10247. DOI: 10.3390/ijms262110247.
100. Racicot K, Mor G. Risks associated with viral infections during pregnancy. J Clin Invest 2017;127(5):1591–1599. DOI: 10.1172/JCI87490.
101. Zerbo O, Iosif AM, Walker C, et al. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (childhood autism risks from genetics and environment) study. J Autism Dev Disord 2013;43(1):25–33. DOI: 10.1007/s10803-012-1540-x.
102. Spann MN, Aydin E, Cheslack-Postava K, et al. Association between maternal c-reactive protein levels in pregnancy and growth trajectories of head circumference during the first year of postnatal life – A secondary data analysis of a case-control study on autism. Pediatr Neonatol 2025;66(3):284–286. DOI: 10.1016/j.pedneo.2024.09.001.
103. Smith SE, Li J, Garbett K, et al. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 2007;27(40):10695–10702. DOI: 10.1523/JNEUROSCI.2178-07.2007.
104. Choi GB, Yim YS, Wong H, et al. The maternal interleukin-17A pathway in mice promotes autism-like phenotypes in offspring. Science 2016;351(6276):933–939. DOI: 10.1126/science.aad0314.
105. Buffington SA, Di Prisco GV, Auchtung TA, et al. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. Cell 2016;165(7):1762–1775. DOI: 10.1016/j.cell.2016.06.001.
106. Vuong HE, Pronovost GN, Williams DW, et al. The maternal microbiome modulates fetal neurodevelopment in mice. Nature 2020;586(7828):281–286. DOI: 10.1038/s41586-020-2745-3.
107. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: Both shared and disorder-specific pathogenesis via perinatal inflammation? Pediatr Res 2011;69(5 Pt 2):26R–33R. DOI: 10.1203/PDR.0b013e318212c196.
108. Assani AD, Boldeanu L, Silosi I, et al. Pregnancy Under Pressure: Oxidative Stress as a Common Thread in Maternal Disorders. Life (Basel) 2025;15(9):1348. DOI: 10.3390/life15091348.
109. Vornic I, Buciu V, Furau CG, et al. Oxidative stress and placental pathogenesis: A contemporary overview of potential biomarkers and emerging therapeutics. Int J Mol Sci 2024;25(22):12195. DOI: 10.3390/ijms252212195.
110. Baroutis D, Sotiropoulou IM, Mantzioros R, et al. Prenatal maternal stress and long-term neurodevelopmental outcomes: A narrative review. J Perinat Med 2025;53(9):1159–1171. DOI: 10.1515/jpm-2025-0297.
111. Bloch MH, Qawasmi A. Omega-3 fatty acid supplementation for the treatment of children with attention-deficit/hyperactivity disorder symptomatology: Systematic review and meta-analysis. J Am Acad Child Adolesc Psychiatry 2011;50(10):991–1000. DOI: 10.1016/j.jaac.2011.06.008.
112. Kurowska A, Ziemichod W, Herbet M, et al. The role of diet as a modulator of the inflammatory process in the neurological diseases. Nutrients 2023;15(6):1436. DOI: 10.3390/nu15061436.
113. Berding K, Vlckova K, Marx W, et al. Diet and the microbiota-gut-brain axis: Sowing the seeds of good mental health. Adv Nutr 2021;12(4):1239–1285. DOI: 10.1093/advances/nmaa181.
114. Beam A, Clinger E, Hao L. Effect of diet and dietary components on the composition of the gut microbiota. Nutrients 2021;13(8):2795. DOI: 10.3390/nu13082795.
115. Schmidt RJ, Hansen RL, Hartiala J, et al. Prenatal vitamins, one-carbon metabolism gene variants, and risk for autism. Epidemiology 2011;22(4):476–485. DOI: 10.1097/EDE.0b013e31821d0e30.
116. Sourander A, Upadhyaya S, Surcel HM, et al. Maternal vitamin D levels during pregnancy and offspring autism spectrum disorder. Biol Psychiatry 2021;90(11):790–797. DOI: 10.1016/j.biopsych.2021.07.012.
117. Dighriri IM, Alsubaie AM, Hakami FM, et al. Effects of omega-3 polyunsaturated fatty acids on brain functions: A systematic review. Cureus 2022;14(10):e30091. DOI: 10.7759/cureus.30091.
118. Beldie LA, Dica CC, Mota M, et al. The interactions between diet and gut microbiota in preventing gestational diabetes mellitus: A narrative review. Nutrients 2024;16(23):4131. DOI: 10.3390/nu16234131.
119. Rodriguez-Cano AM, Gonzalez-Ludlow I, Suarez-Rico BV, et al. Ultra-processed food consumption during pregnancy and its association with maternal oxidative stress markers. Antioxidants (Basel) 2022;11(7):1415. DOI: 10.3390/antiox11071415.
120. Jiang Y, Li Y. The role of nutrition and gut microbiome in childhood brain development and behavior. Front Nutr 2025;12:1590172. DOI: 10.3389/fnut.2025.1590172.
121. Stein A, Pearson RM, Goodman SH, et al. Effects of perinatal mental disorders on the fetus and child. Lancet 2014;384(9956):1800–1819. DOI: 10.1016/S0140-6736(14)61277-0.
122. Goodman SH, Rouse MH, Connell AM, et al. Maternal depression and child psychopathology: A meta-analytic review. Clin Child Fam Psychol Rev 2011;14(1):1–27. DOI: 10.1007/s10567-010-0080-1.
123. Sekar A, Bialas AR, de Rivera H, et al. Schizophrenia risk from complex variation of complement component 4. Nature 2016;530(7589):177–183. DOI: 10.1038/nature16549.
124. Van den Bergh BR, Marcoen A. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year-olds. Child Dev 2004;75(4):1085–1097. DOI: 10.1111/j.1467-8624.2004.00727.x.
125. Laplante DP, Barr RG, Brunet A, et al. Stress during pregnancy affects general intellectual and language functioning in human toddlers. Pediatr Res 2004;56(3):400–410. DOI: 10.1203/01.PDR.0000136281.34035.44.
126. Howard LM, Khalifeh H. Perinatal mental health: A review of progress and challenges. World Psychiatry 2020;19(3):313–327. DOI: 10.1002/wps.20769.
127. Morgan ZEM, Bailey MJ, Trifonova DI, et al. Prenatal exposure to ambient air pollution is associated with neurodevelopmental outcomes at 2 years of age. Environ Health 2023;22(1):11. DOI: 10.1186/s12940-022-00951-y.
128. Cloutier M, Yu C, Talarico R, et al. Prenatal exposure to fine particulate matter components and autism risk in childhood. JAMA Netw Open 2025;8(10):e2538882. DOI: 10.1001/jamanetworkopen.2025.38882.
129. Abarca-Castro EA, Reyes-Lagos JJ, Guzman Ramos K, et al. Fetal development and the air pollution exposome: An integrative perspective of health pathways. Front Cell Neurosci 2025;19:1688437. DOI: 10.3389/fncel.2025.1688437.
130. Saenen ND, Plusquin M, Bijnens E, et al. In utero fine particle air pollution and placental expression of genes in the brain-derived neurotrophic factor signaling pathway: An ENVIRONAGE birth cohort study. Environ Health Perspect 2015;123(8):834–840. DOI: 10.1289/ehp.1408549.

131. Volk HE, Lurmann F, Penfold B, et al. Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatry 2013;70(1):71–77. DOI: 10.1001/jamapsychiatry.2013.266.
132. Sun T, Zheng Z, Yang M, et al. Heavy metal exposure during pregnancy and its association with adverse birth outcomes: A cross-sectional study. Geohealth 2025;9(10):e2025GH001471. DOI: 10.1029/2025GH001471.
133. Rísová V. The pathway of lead through the mother's body to the child. Interdiscip Toxicol 2019;12(1):1–6. DOI: 10.2478/intox-2019-0001.
134. Custodio FB, Andrade A, Guidi LR, et al. Total mercury in commercial fishes and estimation of Brazilian dietary exposure to methylmercury. J Trace Elem Med Biol 2020;62:126641. DOI: 10.1016/j.jtemb.2020.126641.
135. Oken E, Wright RO, Kleinman KP, et al. Maternal fish consumption, hair mercury, and infant cognition in a U.S. cohort. Environ Health Perspect 2005;113(10):1376–1380. DOI: 10.1289/ehp.8041.
136. Tyler CR, Allan AM. The effects of arsenic exposure on neurological and cognitive dysfunction in human and rodent studies: A review. Curr Environ Health Rep 2014;1(2):132–147. DOI: 10.1007/s40572-014-0012-1.
137. Wasserman GA, Liu X, Parvez F, et al. Water arsenic exposure and children's intellectual function in Araihazar, Bangladesh. Environ Health Perspect 2004;112(13):1329–1333. DOI: 10.1289/ehp.6964.
138. Braun JM, Kalkbrenner AE, Calafat AM, et al. Impact of early-life bisphenol A exposure on behavior and executive function in children. Pediatrics 2011;128(5):873–882. DOI: 10.1542/peds.2011-1335.
139. Whyatt RM, Liu X, Rauh VA, et al. Maternal prenatal urinary phthalate metabolite concentrations and child mental, psychomotor, and behavioral development at 3 years of age. Environ Health Perspect 2012;120(2):290–295. DOI: 10.1289/ehp.1103705.
140. ACOG. Committee Opinion No. 723: Guidelines for diagnostic imaging during pregnancy and lactation. Obstet Gynecol 2017;130(4):e210–e216. DOI: 10.1097/AOG.0000000000002355.
141. Ray JG, Vermeulen MJ, Bharatha A, et al. Association between MRI exposure during pregnancy and fetal and childhood outcomes. JAMA 2016;316(9):952–961. DOI: 10.1001/jama.2016.12126.
142. Birks L, Guxens M, Papadopoulou E, et al. Maternal cell phone use during pregnancy and child behavioral problems in five birth cohorts. Environ Int 2017;104:122–131. DOI: 10.1016/j.envint.2017.03.024.
143. Divan HA, Kheifets L, Obel C, et al. Prenatal and postnatal exposure to cell phone use and behavioral problems in children. Epidemiology 2008;19(4):523–529. DOI: 10.1097/EDE.0b013e318175dd47.
144. Reddam A, Sjodin A, Cowell W, et al. Prenatal exposure to polybrominated diphenyl ethers and birth outcomes. Environ Res 2023;216(Pt 4):114830. DOI: 10.1016/j.envres.2022.114830.
145. Bouchard MF, Chevrier J, Harley KG, et al. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ Health Perspect 2011;119(8):1189–1195. DOI: 10.1289/ehp.1003185.
146. Eskenazi B, Chevrier J, Rauch SA, et al. In utero and childhood polybrominated diphenyl ether (PBDE) exposures and neurodevelopment in the CHAMACOS study. Environ Health Perspect 2013;121(2):257–262. DOI: 10.1289/ehp.1205597.
147. Vuong AM, Yolton K, Braun JM, et al. Polybrominated diphenyl ether (PBDE) and poly- and perfluoroalkyl substance (PFAS) exposures during pregnancy and maternal depression. Environ Int 2020;139:105694. DOI: 10.1016/j.envint.2020.105694.
148. Liu B, Lehmler HJ, Ye Z, et al. Exposure to polybrominated diphenyl ethers and risk of all-cause and cause-specific mortality. JAMA Netw Open 2024;7(4):e243127. DOI: 10.1001/jamanetworkopen.2024.3127.
149. Hertz-Picciotto I, Sass JB, Engel S, et al. Organophosphate exposures during pregnancy and child neurodevelopment: Recommendations for essential policy reforms. PLoS Med 2018;15(10):e1002671. DOI: 10.1371/journal.pmed.1002671.
150. Rauh VA, Garfinkel R, Perera FP, et al. Impact of prenatal chlorpyrifos exposure on neurodevelopment in the first 3 years of life among inner-city children. Pediatrics 2006;118(6):e1845–e1859. DOI: 10.1542/peds.2006-0338.
151. Rauh VA, Perera FP, Horton MK, et al. Brain anomalies in children exposed prenatally to a common organophosphate pesticide. Proc Natl Acad Sci U S A 2012;109(20):7871–7876. DOI: 10.1073/pnas.1203396109.
152. Aroniadou-Anderjaska V, Figueiredo TH, de Araujo Furtado M, et al. Mechanisms of organophosphate toxicity and the role of acetylcholinesterase inhibition. Toxics 2023;11(10):866. DOI: 10.3390/toxics11100866.
153. Pearson JN, Patel M. The role of oxidative stress in organophosphate and nerve agent toxicity. Ann N Y Acad Sci 2016;1378(1):17–24. DOI: 10.1111/nyas.13115.
154. Kim DH, Ait Bamai Y, Belova L, et al. Human exposure to persistent and mobile chemicals: A review of sources, internal levels and health implications. Sci Total Environ 2023;893:164764. DOI: 10.1016/j.scitotenv.2023.164764.
155. Tillaut H, Costet N, Monfort C, et al. Occupational exposure to organic solvents during pregnancy and child behavior from early childhood to adolescence. Environ Health 2024;23(1):79. DOI: 10.1186/s12940-024-01120-z.
156. Lerner A, Mainelis G, Hallman W, et al. Managing infectious aerosols to counter engineered pandemics: Current recommendations and future research. Risk Anal 2025;45(10):3045–3078. DOI: 10.1111/risa.70054.
157. Ma Y, Schleck D, Liu J. Prenatal pesticide exposure and adverse reproductive outcomes: Epidemiological evidence and mechanistic insights. J Hazard Mater 2025;494:138792. DOI: 10.1016/j.jhazmat.2025.138792.
158. McMartin KI, Koren G. Exposure to organic solvents: Does it adversely affect pregnancy? Can Fam Physician 1999;45:1671–1673.
159. He S, Wang J, Cao S, et al. Maternal oxidative stress throughout pregnancy and early childhood neurodevelopment at different stages: Insights from a prospective cohort study. BMC Med 2025;23(1):463. DOI: 10.1186/s12916-025-04297-3.
160. Alhowail A. Mechanisms underlying cognitive impairment induced by prenatal alcohol exposure. Brain Sci 2022;12(12):1667. DOI: 10.3390/brainsci12121667.
161. Suter MA, Aagaard KM. The impact of tobacco chemicals and nicotine on placental development. Prenat Diagn 2020;40(9):1193–1200. DOI: 10.1002/pd.5660.
162. Hviid A, Melbye M, Pasternak B. Use of selective serotonin reuptake inhibitors during pregnancy and risk of autism. N Engl J Med 2013;369(25):2406–2415. DOI: 10.1056/NEJMoa1301449.
163. Cox RC. Associations between sleep and circadian rhythm disruption and perinatal anxiety. NPJ Biol Timing Sleep 2025;2(1):33. DOI: 10.1038/s44323-025-00051-3.
164. Rute-Larrieta C, Mota-Catedra G, Carmona-Torres JM, et al. Physical activity during pregnancy and risk of gestational diabetes mellitus: A meta-review. Life (Basel) 2024;14(6):755. DOI: 10.3390/life14060755.
165. Pirie PL, Lando H, Curry SJ, et al. Tobacco, alcohol, and caffeine use and cessation in early pregnancy. Am J Prev Med 2000;18(1):54–61. DOI: 10.1016/s0749-3797(99)00088-4.
166. Phoswa WN, Khaliq OP. The role of oxidative stress in hypertensive disorders of pregnancy (preeclampsia, gestational hypertension) and metabolic disorder of pregnancy (gestational diabetes mellitus). Oxid Med Cell Longev 2021;2021:5581570. DOI: 10.1155/2021/5581570.
167. Maher GM, O'Keeffe GW, Kearney PM, et al. Association of hypertensive disorders of pregnancy with risk of neurodevelopmental disorders in offspring: A systematic review and meta-analysis. JAMA Psychiatry 2018;75(8):809–819. DOI: 10.1001/jamapsychiatry.2018.0854.
168. Stubert J, Hinz B, Berger R. The role of acetylsalicylic acid in the prevention of pre-eclampsia, fetal growth restriction, and preterm birth. Dtsch Arztebl Int 2023;120(37):617–626. DOI: 10.3238/arztebl.m2023.0133.
169. Lammertink F, Vinkers CH, Tataranno ML, et al. Premature birth and developmental programming: Mechanisms of resilience and vulnerability. Front Psychiatry 2020;11:531571. DOI: 10.3389/fpsyt.2020.531571.

170. Franz AP, Bolat GU, Bolat H, et al. Attention-deficit/hyperactivity disorder and very preterm/very low birth weight: A meta-analysis. Pediatrics 2018;141(1):e20171645. DOI: 10.1542/peds.2017-1645.
171. Miller SL, Huppi PS, Mallard C. The consequences of fetal growth restriction on brain structure and neurodevelopmental outcome. J Physiol 2016;594(4):807–823. DOI: 10.1113/JP271402.
172. Sacchi C, Marino C, Nosarti C, et al. Association of intrauterine growth restriction and small for gestational age status with childhood cognitive outcomes: A systematic review and meta-analysis. JAMA Pediatr 2020;174(8):772–781. DOI: 10.1001/jamapediatrics.2020.1097.
173. Shankaran S, Laptook AR, McDonald SA, et al. Acute perinatal sentinel events, neonatal brain injury pattern, and outcome of infants undergoing a trial of hypothermia for neonatal hypoxic-ischemic encephalopathy. J Pediatr 2017;180:275–278.e2. DOI: 10.1016/j.jpeds.2016.09.026.
174. Shankaran S, Pappas A, McDonald SA, et al. Childhood outcomes after hypothermia for neonatal encephalopathy. N Engl J Med 2012;366(22):2085–2092. DOI: 10.1056/NEJMoa1112066.
175. Mitchell CM, Mazzoni C, Hogstrom L, et al. Delivery mode affects stability of early infant gut microbiota. Cell Rep Med 2020;1(9):100156. DOI: 10.1016/j.xcrm.2020.100156.
176. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107(26):11971–11975. DOI: 10.1073/pnas.1002601107.
177. Lupu VV, Miron IC, Raileanu AA, et al. Difficulties in adaptation of the mother and newborn via cesarean section versus natural birth—A narrative review. Life (Basel) 2023;13(2):300. DOI: 10.3390/life13020300.
178. Jantzie LL. Placental mediated mechanisms of perinatal brain injury. Exp Neurol 2022;358:114229. DOI: 10.1016/j.expneurol.2022.114229.
179. Wu Y, De Asis-Cruz J, Limperopoulos C. Brain structural and functional outcomes in the offspring of women experiencing psychological distress during pregnancy. Mol Psychiatry 2024;29(7):2223–2240. DOI: 10.1038/s41380-024-02449-0.
180. Countouris M, Mahmoud Z, Cohen JB, et al. Hypertension in pregnancy and postpartum: Current standards and opportunities to improve care. Circulation 2025;151(7):490–507. DOI: 10.1161/CIRCULATIONAHA.124.073302.
181. Bernstein PS, Martins JN Jr, Barton JR, et al. National partnership for maternal safety: Consensus bundle on severe hypertension during pregnancy and the postpartum period. Obstet Gynecol 2017;130(2):347–357. DOI: 10.1097/AOG.0000000000002115.
182. Herzberg MP, Smyser CD. Prenatal social determinants of health: Narrative review of maternal environments and neonatal brain development. Pediatr Res 2024;96(6):1417–1428. DOI: 10.1038/s41390-024-03345-7.
183. Aizer A. Poverty, violence and health: The impact of domestic violence during pregnancy on newborn health. J Hum Resour 2011;46(3):518–538. DOI: 10.1353/jhr.2011.0024.
184. Alhusen JL, Ray E, Sharps P, Bullock L. Intimate partner violence during pregnancy: Maternal and neonatal outcomes. J Womens Health (Larchmt) 2015;24(1):100–106. DOI: 10.1089/jwh.2014.4872.
185. Mueller I, Tronick E. Early life exposure to violence: Developmental consequences on brain and behavior. Front Behav Neurosci 2019;13:156. DOI: 10.3389/fnbeh.2019.00156.
186. Woods RM, Lorusso JM, Fletcher J, et al. Maternal immune activation and role of placenta in the prenatal programming of neurodevelopmental disorders. Neuronal Signal 2023;7(2):NS20220064. DOI: 10.1042/NS20220064.
187. Yong Q, Zhao C, Xia L, et al. Maternal immune activation and neurodevelopmental disorders: Integrating molecular, cellular and systems mechanisms. Neuropsychiatr Dis Treat 2025;21:2575–2594. DOI: 10.2147/NDT.S533813.
188. Clougherty JE, Kubzansky LD. A framework for examining social stress and susceptibility to air pollution in respiratory health. Environ Health Perspect 2009;117(9):1351–1358. DOI: 10.1289/ehp.0900612.
189. Silverman JG, Decker MR, Reed E, Raj A. Intimate partner violence around the time of pregnancy: Association with breastfeeding behavior. J Womens Health (Larchmt) 2006;15(8):934–940. DOI: 10.1089/jwh.2006.15.934.
190. Grote NK, Bridge JA, Gavin AR, et al. A meta-analysis of depression during pregnancy and the risk of preterm birth, low birth weight, and intrauterine growth restriction. Arch Gen Psychiatry 2010;67(10):1012–1024. DOI: 10.1001/archgenpsychiatry.2010.111.
191. Noble KG, Houston SM, Brito NH, et al. Family income, parental education and brain structure in children and adolescents. Nat Neurosci 2015;18(5):773–778. DOI: 10.1038/nn.3983.
192. Norbom LB, Rokicki J, Eilertsen EM, et al. Parental education and income are linked to offspring cortical brain structure and psychopathology at 9–11 years. JCPP Adv 2024;4(1):e12220. DOI: 10.1002/jcv2.12220.
193. Ailshire J, Karraker A, Clarke P. Neighborhood social stressors, fine particulate matter air pollution, and cognitive function among older U.S. adults. Soc Sci Med 2017;172:56–63. DOI: 10.1016/j.socscimed.2016.11.019.
194. Liu YZ, Wang YX, Jiang CL. Inflammation: The common pathway of stress-related diseases. Front Hum Neurosci 2017;11:316. DOI: 10.3389/fnhum.2017.00316.
195. Perera FP, Wang S, Rauh V, et al. Prenatal exposure to air pollution, maternal psychological distress, and child behavior. Pediatrics 2013;132(5):e1284–e1294. DOI: 10.1542/peds.2012-3844.
196. Golonka RM, Xiao X, Abokor AA, et al. Altered nutrient status reprograms host inflammation and metabolic health via gut microbiota. J Nutr Biochem 2020;80:108360. DOI: 10.1016/j.jnutbio.2020.108360.
197. Monthe-Dreze C, Penfield-Cyr A, Smid MC, et al. Maternal pre-pregnancy obesity attenuates response to omega-3 fatty acids supplementation during pregnancy. Nutrients 2018;10(12):1908. DOI: 10.3390/nu10121908.
198. Al-Mutawtah M, Campbell E, Kubis HP, et al. Women's experiences of social support during pregnancy: A qualitative systematic review. BMC Pregnancy Childbirth 2023;23(1):782. DOI: 10.1186/s12884-023-06089-0.
199. Mercier O, Fu SY, Filler R, et al. Interventions for intimate partner violence during the perinatal period: A scoping review: A systematic review. Campbell Syst Rev 2024;20(3):e1423. DOI: 10.1002/cl2.1423.
200. Sabri B, Tharmarajah S, Njie-Carr VPS, et al. Safety planning with marginalized survivors of intimate partner violence: Challenges of conducting safety planning intervention research with marginalized women. Trauma Violence Abuse 2022;23(5):1728–1751. DOI: 10.1177/15248380211013136.
201. Leighton C, Botto A, Silva JR, et al. Vulnerability or sensitivity to the environment? Methodological issues, trends, and recommendations in gene-environment interactions research in human behavior. Front Psychiatry 2017;8:106. DOI: 10.3389/fpsyt.2017.00106.
202. Wu W, Zhou X, Jiang Z, et al. Noninvasive fetal genotyping of single nucleotide variants and linkage analysis for prenatal diagnosis of monogenic disorders. Hum Genomics 2022;16(1):28. DOI: 10.1186/s40246-022-00400-4.
203. Williams M, Zhang Z, Nance E, et al. Maternal inflammation results in altered tryptophan metabolism in rabbit placenta and fetal brain. Dev Neurosci 2017;39(5):399–412. DOI: 10.1159/000471509.
204. Douma EH, de Kloet ER. Stress-induced plasticity and functioning of ventral tegmental dopamine neurons. Neurosci Biobehav Rev 2020;108:48–77. DOI: 10.1016/j.neubiorev.2019.10.015.
205. Sakai J. Core concept: How synaptic pruning shapes neural wiring during development and, possibly, in disease. Proc Natl Acad Sci U S A 2020;117(28):16096–16099. DOI: 10.1073/pnas.2010281117.
206. Ferro A, Auguste YSS, Cheadle L. Microglia, cytokines, and neural activity: Unexpected interactions in brain development and function. Front Immunol 2021;12:703527. DOI: 10.3389/fimmu.2021.703527.
207. Nandi S, de Deus JL, Faborode OS, et al. Synaptic pruning by microglia: Lessons from genetic studies in mice. Dev Neurosci 2025;47(5):362–382. DOI: 10.1159/000541379.

208. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic pruning by microglia is necessary for normal brain development. Science 2011;333(6048):1456–1458. DOI: 10.1126/science.1202529.
209. Wohrer F, Ngo H, DiDomenico J, et al. Potentially modifiable risk and protective factors affecting mental and emotional wellness in pregnancy. Front Hum Neurosci 2024;18:1323297. DOI: 10.3389/fnhum.2024.1323297.
210. Steele-Baser M, Brown AL, D'Angelo DV, et al. Intimate partner violence and pregnancy and infant health outcomes—Pregnancy Risk Assessment Monitoring System, nine U.S. jurisdictions, 2016–2022. MMWR Morb Mortal Wkly Rep 2024;73(48):1093–1098. DOI: 10.15585/mmwr.mm7348a1.
211. Satapathy P, Gaidhane AM, Vadia N, et al. Exposure to violence and risk of hypertensive disorders in pregnancy: Systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol X 2025;26:100398. DOI: 10.1016/j.eurox.2025.100398.
212. Ramirez SI. Prenatal care: An evidence-based approach. Am Fam Physician 2023;108(2):139–150.
213. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. N Engl J Med 1992;327(26):1832–1835. DOI: 10.1056/NEJM199212243272602.
214. Davis EP, Narayan AJ. Pregnancy as a period of risk, adaptation, and resilience for mothers and infants. Dev Psychopathol 2020;32(5):1625–1639. DOI: 10.1017/S0954579420001121.
215. Lugo-Candelas C, Talati A, Glickman C, et al. Maternal mental health and offspring brain development: An umbrella review of prenatal interventions. Biol Psychiatry 2023;93(10):934–941. DOI: 10.1016/j.biopsych.2023.01.026.
216. Marshall NE, Abrams B, Barbour LA, et al. The importance of nutrition in pregnancy and lactation: Lifelong consequences. Am J Obstet Gynecol 2022;226(5):607–632. DOI: 10.1016/j.ajog.2021.12.035.
217. Na X, Mackean PP, Cape GA, et al. Maternal nutrition during pregnancy and offspring brain development: Insights from neuroimaging. Nutrients 2024;16(19):3337. DOI: 10.3390/nu16193337.
218. Stapleton LR, Schetter CD, Westling E, et al. Perceived partner support in pregnancy predicts lower maternal and infant distress. J Fam Psychol 2012;26(3):453–463. DOI: 10.1037/a0028332.
219. Hawken T, Turner-Cobb J, Barnett J. Coping and adjustment in caregivers: A systematic review. Health Psychol Open 2018;5(2):2055102918810659. DOI: 10.1177/2055102918810659.
220. Vazquez-Lara MD, Ruger-Navarrete A, Mohamed-Abdel-Lah S, et al. The impact of mindfulness programmes on anxiety, depression and stress during pregnancy: A systematic review and meta-analysis. Healthcare (Basel) 2025;13(12):1378. DOI: 10.3390/healthcare13121378.
221. Gomez Y, Nakaki A, Conti A, et al. Mindfulness-based stress reduction intervention during pregnancy changes maternal brain. Sci Rep 2025;15(1):21929. DOI: 10.1038/s41598-025-07787-9.
222. Walinder R, Gunnarsson K, Runeson R, et al. Physiological and psychological stress reactions in relation to classroom noise. Scand J Work Environ Health 2007;33(4):260–266. DOI: 10.5271/sjweh.1141.
223. Chen X, Deng YF, Fu CF, et al. A physical activity counseling intervention to promote health among pregnant women: A study protocol of randomized clinical trial. BMC Pregnancy Childbirth 2025;25(1):264. DOI: 10.1186/s12884-025-07268-x.
224. Chae SA, Son JS, Du M. Prenatal exercise in fetal development: A placental perspective. FEBS J 2022;289(11):3058–3071. DOI: 10.1111/febs.16173.
225. Pizzorno J. Toxin exposure reduction. Integr Med (Encinitas) 2017;16(6):8–10.
226. Aguilera J, Konvinse K, Lee A, et al. Air pollution and pregnancy. Semin Perinatol 2023;47(8):151838. DOI: 10.1016/j.semperi.2023.151838.
227. Nigra AE, Bloomquist TR, Rajeev T, et al. Public water arsenic and birth outcomes in the environmental influences on child health outcomes cohort. JAMA Netw Open 2025;8(6):e2514084. DOI: 10.1001/jamanetworkopen.2025.14084.
228. Pogodina C, Brunner Huber LR, Racine EF, et al. Smoke-free homes for smoke-free babies: The role of residential environmental tobacco smoke on low birth weight. J Community Health 2009;34(5):376–382. DOI: 10.1007/s10900-009-9169-1.
229. Jinesh S, Aditi P. Health implications of microplastic exposure in pregnancy and early childhood: A systematic review. Int J Womens Health 2025;17:2805–2818. DOI: 10.2147/IJWH.S497366.
230. Pan K, Xu J, Li F, et al. The association between mercury exposure during pregnancy and adverse birth outcomes: A systematic review and meta-analysis. Environ Res 2025;264(Pt 1):120357. DOI: 10.1016/j.envres.2024.120357.
231. Rolfo A, Nuzzo AM, De Amicis R, et al. Fetal-maternal exposure to endocrine disruptors: Correlation with diet intake and pregnancy outcomes. Nutrients 2020;12(6):1744. DOI: 10.3390/nu12061744.
232. Li Y, Wang X, Zhu Q, et al. Organophosphate flame retardants in pregnant women: Sources, occurrence, and potential risks to pregnancy outcomes. Environ Sci Technol 2023;57(18):7109–7128. DOI: 10.1021/acs.est.2c06503.
233. Murphy M, McHugh S, O'Keeffe LM, et al. Preventive health counselling during antenatal care using the pregnancy risk assessment monitoring system (PRAMS) in Ireland. BMC Pregnancy Childbirth 2020;20(1):98. DOI: 10.1186/s12884-020-2756-y.
234. Villar J, Bergsjo P. Scientific basis for the content of routine antenatal care. I. Philosophy, recent studies, and power to eliminate or alleviate adverse maternal outcomes. Acta Obstet Gynecol Scand 1997;76(1):1–14. DOI: 10.3109/00016349709047778.
235. Holder K, Feinglass J, Niznik C, et al. Use of electronic patient messaging by pregnant patients receiving prenatal care at an academic health system: Retrospective cohort study. JMIR Mhealth Uhealth 2024;12:e51637. DOI: 10.2196/51637.
236. Devkota R, Khan GM, Alam K, et al. Impacts of counseling on knowledge, attitude and practice of medication use during pregnancy. BMC Pregnancy Childbirth 2017;17(1):131. DOI: 10.1186/s12884-017-1316-6.
237. Zulman DM, Vijan S, Omenn GS, et al. The relative merits of population-based and targeted prevention strategies. Milbank Q 2008;86(4):557–580. DOI: 10.1111/j.1468-0009.2008.00534.x.
238. Sanderson D, Braganza S, Philips K, et al. Increasing warm handoffs: Optimizing community based referrals in primary care using QI methodology. J Prim Care Community Health 2021;12:21501327211023883. DOI: 10.1177/21501327211023883.
239. Premkumar A, Mele L, Casey BM, et al. Relationship between maternal economic vulnerability and childhood neurodevelopment at 2 and 5 years of life. Obstet Gynecol 2021;138(3):379–388. DOI: 10.1097/AOG.0000000000004503.
240. Ruggieri S, Drago G, Panunzi S, et al. The influence of sociodemographic factors, lifestyle, and risk perception on dietary patterns in pregnant women living in highly contaminated areas: Data from the NEHO birth cohort. Nutrients 2022;14(17):3489. DOI: 10.3390/nu14173489.
241. Wesolowska E, Jankowska A, Trafalska E, et al. Sociodemographic, lifestyle, environmental and pregnancy-related determinants of dietary patterns during pregnancy. Int J Environ Res Public Health 2019;16(5):0754. DOI: 10.3390/ijerph16050754.
242. Souza JP, Day LT, Rezende-Gomes AC, et al. A global analysis of the determinants of maternal health and transitions in maternal mortality. Lancet Glob Health 2024;12(2):e306–e316. DOI: 10.1016/S2214-109X(23)00468-0.
243. Woods WA, Watson M, Ranaweera S, et al. Under-representation of low and middle income countries (LMIC) in the research literature: Ethical issues arising from a survey of five leading medical journals: Have the trends changed? Glob Public Health 2023;18(1):2229890. DOI: 10.1080/17441692.2023.2229890.
244. Singh M, Shekhar C, Gupta J, et al. Exploring the shifting landscape of delayed motherhood in India: A comprehensive analysis using joinpoint and age-period-cohort analysis. BMC Womens Health 2025;25(1):563. DOI: 10.1186/s12905-025-04104-4.
245. Barros AJ, Matijasevich A, Santos IS, et al. Child development in a birth cohort: Effect of child stimulation is stronger in less educated mothers. Int J Epidemiol 2010;39(1):285–294. DOI: 10.1093/ije/dyp272.

246. Lassi ZS, Padhani ZA, Rabbani A, et al. Effects of nutritional interventions during pregnancy on birth, child health and development outcomes: A systematic review of evidence from low- and middle-income countries. Campbell Syst Rev 2021;17(2):e1150. DOI: 10.1002/cl2.1150.
247. Kent DM, Nelson J, Dahabreh IJ, et al. Risk and treatment effect heterogeneity: Re-analysis of individual participant data from 32 large clinical trials. Int J Epidemiol 2016;45(6):2075–2088. DOI: 10.1093/ije/dyw118.
248. Wood ME, Lupattelli A, Palmsten K, et al. Longitudinal methods for modeling exposures in pharmacoepidemiologic studies in pregnancy. Epidemiol Rev 2022;43(1):130–146. DOI: 10.1093/epirev/mxab002.
249. Andrawus M, Sharvit L, Atzmon G. Epigenetics and pregnancy: Conditional snapshot or rolling event. Int J Mol Sci 2022;23(20):12698. DOI: 10.3390/ijms232012698.
250. Basak S, Mallick R, Navya Sree B, et al. Placental epigenome impacts fetal development: Effects of maternal nutrients and gut microbiota. Nutrients 2024;16(12):1860. DOI: 10.3390/nu16121860.
251. Handy DE, Castro R, Loscalzo J. Epigenetic modifications: Basic mechanisms and role in cardiovascular disease. Circulation 2011;123(19):2145–2156. DOI: 10.1161/CIRCULATIONAHA.110.956839.
252. Rozek LS, Dolinoy DC, Sartor MA, et al. Epigenetics: Relevance and implications for public health. Annu Rev Public Health 2014;35:105–122. DOI: 10.1146/annurev-publhealth-032013-182513.
253. Glaab E, Rauschenberger A, Banzi R, et al. Biomarker discovery studies for patient stratification using machine learning analysis of omics data: A scoping review. BMJ Open 2021;11(12):e053674. DOI: 10.1136/bmjopen-2021-053674.
254. Landeiro F, Silva M, Moura CVE, et al. Human-centered design and maternity care: Is this a possible interplay?-a systematic review. BMC Pregnancy Childbirth 2025;25(1):261. DOI: 10.1186/s12884-024-07119-1.
255. Doherty E, Kingsland M, Wolfenden L, et al. Implementation strategies to improve preconception and antenatal care for tobacco smoking, alcohol consumption and weight management: A systematic review protocol. Syst Rev 2019;8(1):285. DOI: 10.1186/s13643-019-1193-3.
256. Fitzgerald E, Hor K, Drake AJ. Maternal influences on fetal brain development: The role of nutrition, infection and stress, and the potential for intergenerational consequences. Early Hum Dev 2020;150:105190. DOI: 10.1016/j.earlhumdev.2020.105190.
257. Tooley UA, Latham A, Kenley JK, et al. Prenatal environment is associated with the pace of cortical network development over the first three years of life. Nat Commun 2024;15(1):7932. DOI: 10.1038/s41467-024-52242-4.
258. Gonzalez FF, Ferriero DM. Neuroprotection in the newborn infant. Clin Perinatol 2009;36(4):859–880, vii. DOI: 10.1016/j.clp.2009.07.013.
259. Entringer S, Buss C, Swanson JM, et al. Fetal programming of body composition, obesity, and metabolic function: The role of intrauterine stress and stress biology. J Nutr Metab 2012;2012:632548. DOI: 10.1155/2012/632548.
260. Sinnott-Armstrong N, Fields S, Roth F, et al. Understanding genetic variants in context. Elife 2024;13:88231. DOI: 10.7554/eLife.88231.
261. Geese F, Schmitt KU. Interprofessional collaboration in complex patient care transition: A qualitative multi-perspective analysis. Healthcare (Basel) 2023;11(3):359. DOI: 10.3390/healthcare11030359.

CASE REPORT

Case Report: A Congenital Pouch Colon with Anorectal Malformation and Associated Anomalies

Atul K Khare[1], Ramesh C Tanger[1], Aditya J Baindur[1]

Received on: 21 August 2025; *Accepted on*: 23 December 2025; *Published on*: 15 January 2026

Abstract

Background: Congenital pouch colons (CPCs) are rare congenital malformations that are seen in the Indian Subcontinent and the surrounding region. These patients show major dilatations in one/more segments of the entire colon that are often associated with an anorectal malformation (ARM) and/or a fistulous communication with the distal urogenital tract. Many also show vesicoureteral reflux, hydronephrosis, hypospadias, a bicornuate/septate uterus, an absent/double appendix, a Meckel's diverticulum, rectal atresia, sacral agenesis, and congenital heart defects. This case shows that timely identification and management of CPCs can improve the outcome of these patients.

Case presentation: We recently treated a four-day-old full-term, small-for-gestation female infant weighing 2.3 kg, who was presented with vomiting, abdominal distension, and had been passing stools through a single cloacal opening. An erect radiogram showed a massive colonic gas shadow on the left side that displaced the whole small bowel towards the right. An inverted X-ray showed a high anorectal malformation. Cystoscopy showed a constricted neck of the bladder and a bicornuate uterus. Hysteroscopy, and then an exploratory laparotomy confirmed the presence of a uterocolonic fistula, a massivelydilated pouch colon, a double appendix, and a Meckel's diverticulum. The fistula was ligated, and a pouch ostomy was done. A review of the case showed that she had a type-2 CPC with cloaca, a colon-uterine fistula, a bicornuate uterus, double appendix, and a Meckel's diverticulum. The infant stabilized over the next few days and was discharged from the hospital. She is being followed up and is progressing well.

Conclusion: Congenital pouch colons are complex congenital anomalies that can benefit from early identification and proper management. The condition is seen more frequently in males and is promptly diagnosed during the neonatal period due to abdominal distention, absence of anus, and intestinal obstruction. It is managed surgically depending on its type, with timely diagnosis and management. Congenital pouch colon is a rare but important condition where timely diagnosis and management can improve the outcome.

Keywords: Anorectal malformation, Appendices epiploicae, Bicornuate uterine, Bicornuate uterus, *C7orf57*, *C9orf84*, Case report, Cloaca, Colon-uterine fistula, Congenital heart defects, Didelphys uterus, Double appendix, *FGFR4*, Haustrations, *HLA-DRB5*, Hydronephrosis, Hypospadias, Infant, Meckel's diverticulum, Neonate, *NOTCH2NLA*, Pouch ostomy, Pubococcygeal line, Rectal atresia, Sacral agenesis, Septate uterus, Taeniae, Type-2 CPC, Ureterohydronephrosis.

Newborn (2025): 10.5005/jp-journals-11002-0146

Introduction

Congenital pouch colons (CPCs) show pouch-like dilatation of a part or the whole colon, which communicates distally with the urogenital tract by a large fistula.[1–3] These are frequently associated with anorectal malformations (ARMs), double appendix, and sometimes, also with a Meckel's diverticulum.[4,5]

Congenital pouch colons have been reported almost exclusively from South Asia, particularly India (90%), Pakistan (8–10%), and Bangladesh (2%).[2,6–8] In India, CPCs comprise about 5–15% of all ARMs.[9] Only sporadic cases have been seen in other parts of the world.[10] Congenital pouch colons are seen more frequently in males than in females (3–7:1).[7] Those in female infants are often seen associated with a cloaca, colon-uterine fistula, and a bicornuate uterus.[10] Here, we report an infant with such findings.

Case Description

We recently treated a three-day-old full-term, small-for-date female infant weighing 2.3 kg born after a normal vaginal birth. She was presented with vomiting, a distended abdomen, and passage of stools from a single cloacal opening (Fig. 1). The perineal examination showed a flat perineum with poorly developed buttocks. Laboratory evaluation showed a normal hemogram and blood biochemistry profile.

An erect radiograph of the abdomen showed a single dilated intestinal loop with an air-fluid level on the left side of the abdomen; it occupied >50% of the abdominal width and terminated in a supra-levator position. The small bowel was displaced to the other side. The inverted X-ray showed the pouch as proximal to the pubococcygeal line, consistent with a high ARM. A prone cross-table lateral X-ray also showed the gas shadow to be above the pubococcygeal line.

[1]Department of Pediatric Surgery, SMS Medical College, Jaipur, Rajasthan, India

Corresponding Author: Aditya J Baindur, Department of Pediatric Surgery, SMS Medical College, Jaipur, Rajasthan, India, Phone: +91 8839523630, e-mail: draditya jbaindur@gmail.com

How to cite this article: Khare AK, Tanger RC, Baindur AJ. Case Report: A Congenital Pouch Colon with Anorectal Malformation and Associated Anomalies. Newborn 2025;4(4):221–224.

Source of support: Nil

Conflict of interest: None

Patient consent statement: A written informed consent was obtained from the patient for the publication of details, which can include photographs and/or videos and/or case history to be published in any printed/online journals.

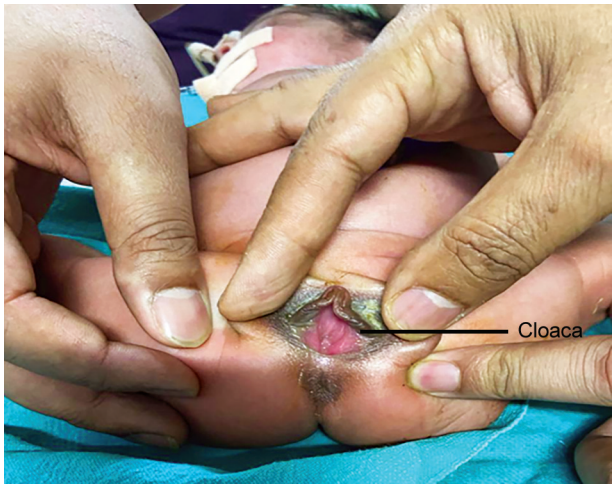


Fig. 1: Physical examination at admission showed a single cloacal opening in the perineum, through which the infant was passing both urine and stool

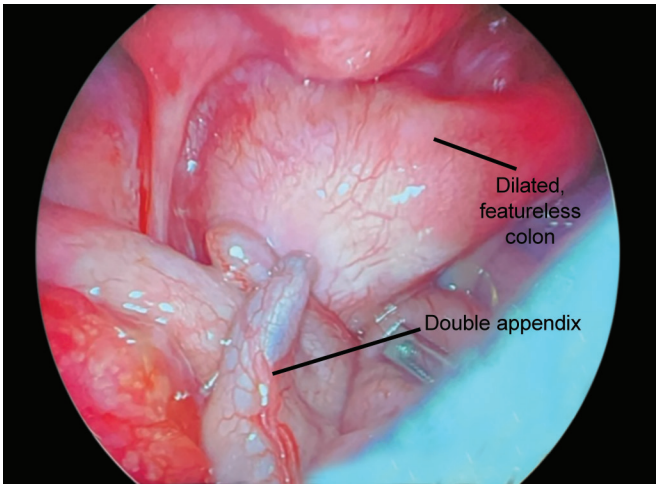


Fig. 3: Diagnostic hysteroscopy showed a dilated colon and a double appendix

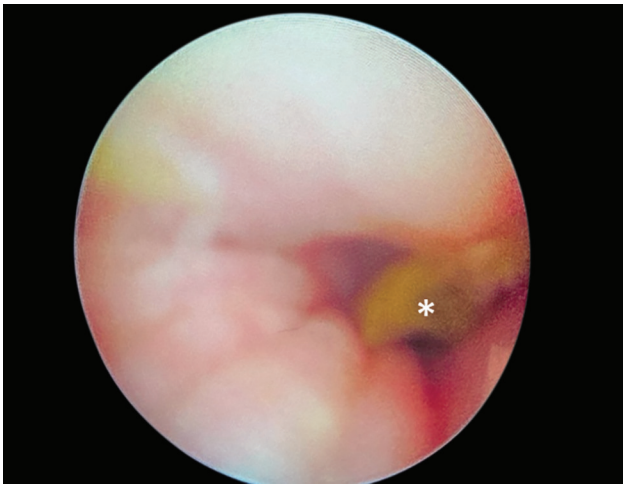


Fig. 2: Hysteroscopy showed a colouterine fistula. Stool can be seen at the opening of the fistula (marked by an asterisk, *)

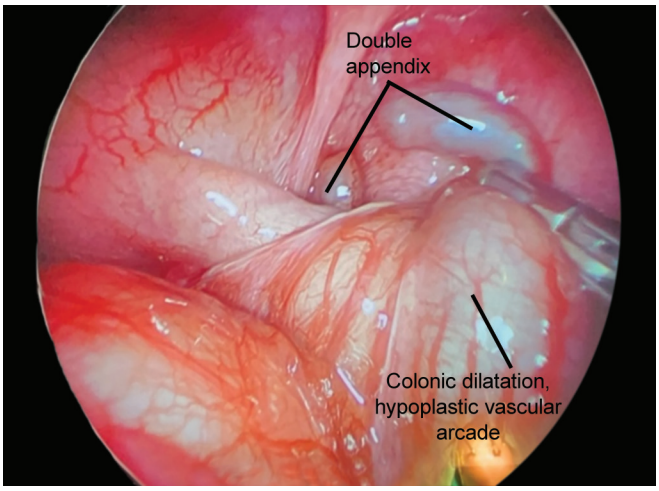


Fig. 4: An exploratory laparotomy confirmed the findings of colonic dilatation, double appendix, and hypoplastic vascular arcades

Ultrasonography of the abdomen and pelvis showed a bicornuate uterus. The large bowel was hugely dilated. A single perineal opening was seen.

Management

After adequate resuscitation, a nasogastric tube was placed. A cystoscopy was performed; the bladder wall and ureteric openings appeared normal. The overall volume of the bladder was larger than normal, and the neck was constricted. A urogenital sinus was seen, and the uterus was filled with stool. The hysteroscopy showed a bicornuate uterus with a large colouterine fistula where fecal matter could be seen (Fig. 2).

Diagnostic laparoscopy showed the whole colon as hugely dilated, and it showed no haustrations, taeniae, or appendices epiploicae. The cecum drained into this dilated colon. A double appendix (Fig. 3) was seen. A Meckel's diverticulum was noted proximal to the ileocecal junction. The other end of the colon opened into the uterus with a large colon-uterine fistula.

An exploratory laparotomy was performed under general anesthesia through a transverse incision in the left lower quadrant. The whole colon was dilated like a 16 × 5 cm pouch and showed no haustrations, taeniae, or appendices epiploicae. About 10 cm proximal to the ileocecal junction, a Meckel's diverticulum, about 2 × 0.5 cm in size, was seen. The cecum drained into the colonic pouch, and two appendices, each about 3 × 0.5 cm in size, were notable adjacent to the ileocecal junction. A large colouterine fistula could be seen proximally adjacent to the ileal opening. The pouch had minimal meconium and gas, as it was draining into the colouterine fistula. The pouch colon was attached by a very short mesentery, and the vascular arcade also appeared hypoplastic (Fig. 4).

We ligated the fistula at the lowest possible end. An end-pouch ostomy was created around 5 cm proximal to the lower end of the fistula. The pouch opening was kept at a minimal size to prevent prolapse, just sufficient for decompression (Fig. 5).

In the postoperative period, the stoma began functioning after 3 days. We started maternal feedings and were able to discharge

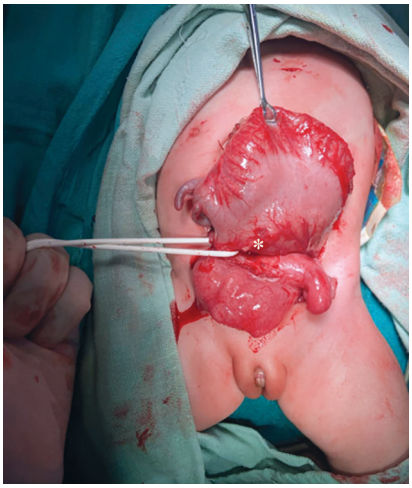


Fig. 5: Exploratory laparotomy showed a large colouterine fistula (marked by an asterisk, *)

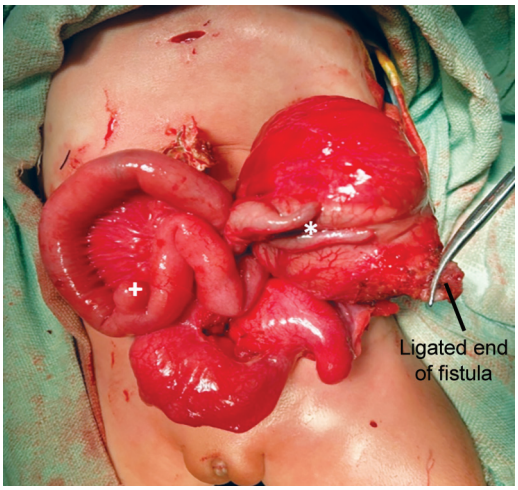


Fig. 6: Exploratory laparotomy showed a double appendix (marked by an asterisk, *) and Meckel's diverticulum (marked by a plus sign, +) after ligation of the fistula (ligated end is labeled)

home on postoperative day 5. Parents were trained for washing the pouch, were requested to follow up in the clinic every 2 weeks. The child is recovering well, and we are following up closely to decide on further procedures (Fig. 6).

Discussion

Congenital pouch colons were first described by Spriggs in 1912 in a specimen with an absent left half of the colon and rectum.[11] The term CPC syndrome was proposed by Narsimha Rao in 1984.[12] Several groups have attempted to classify CPCs since then[1,7,8] The first attempt at classification was made by Chadha and his team in 1984.[6] The investigators tried to recognize four subtypes (types I–IV) based on the length of the normal colon proximal to the colonic pouch, with types I and II being the most severe ones, and type IV CPC being the most common presentation. In type I, the normal colon is absent, and the ileum opens directly into the colonic pouch. In type II, the ileum opens into a short segment of the cecum, which then opens into the colonic pouch. In type III, there is a significant amount of normal colon between the ileum and the colonic pouch. Type IV is characterized by the presence of a nearly normal colon with only the terminal portion of the colon ending into the pouch.

The best recognized classification is the one proposed by Saxena and Mathur with types 1–5.[13] They proposed that there is a fifth category of patients; this type V shows a double pouch colon with normal segments between the pouches. However, many clinicians find that a simple distinction between complete CPC and incomplete CPC, with the extent of involvement, is all that is needed for management and prognostication. Some exceptions do get recognized, as one described by Nascimben et al.[14] They treated a 1530-gram preterm syndromic female with a type II CPC, a complex cloaca, and a retrovesical didelphus uterus. A preoperative cystoscopy identified a urogenital sinus with an anterior bladder and a posterior vagina.

The pathogenesis and embryology of CPC are not well understood. These conditions seem to be related to aborted development of the hindgut, with obliteration of the inferior mesenteric artery and maldevelopment of the terminal midgut early in fetal life.[15] Others have associated a vascular compromise in the anorectal fold and adjacent hindgut. Most cases of CPC are sporadic with no familial inheritance. However, some dietary and environmental factors may have a role; iodine and vitamin B deficiency, pesticides, and fungicides have been implicated.[6] Genetic studies have not provided strong causal evidence so far. There are some possible associations, such as *C7orf57*, *C9orf84*, *FGFR4*, *HLA-DRB5*, and *NOTCH2NLA*.[16] Some other mutations affecting the Wnt, NOTCH, and Hedgehog pathways have also been identified as possibilities.[17,18]

As for the diagnosis, it is typically done by an imaging study. Sometimes, anteroposterior and lateral radiographs may be adequate if they show a large air-fluid level with small bowel loops displaced to the right.[8] Patients with a colovesical fistula may show gas in the bladder or meconium; ammonium hydrogen urate calcifications can be found in the colon. Others with a fistula may show low colon pH, urine, and stasis.[19] In most cases, CPC syndrome is challenging to diagnose due to non-specific symptoms and non-definitive imaging studies. Neonates with an ARM and signs of intestinal obstruction should raise suspicion.[1,9,20] Other strong associations with CPC syndrome include cardiac, vertebral, and genitourinary anomalies; echocardiography, vertebral X-ray, and micturating cystourethrogram can be useful.[20]

Surgical management and outcomes of CPC mainly depend on the subtype, the length of the affected colon, the type of perineum, and anorectal muscle complexity, as well as other congenital associations. A congenital pouch colon is considered incomplete if the length of the normal colon is adequate for performing a pull-through without the need for coloplasty.[14] In incomplete CPC, surgery is the preferred choice with primary excision of the colonic pouch with associated procedures, as needed: Ileostomy; window colostomy; end colostomy; transverse colostomy, and ligation of fistula. These would be followed by definitive surgery with endorectal pull-through and coloanal anastomosis, preserving the native anal sphincter complex.[20,21] The ligation of fistulas is usually preferred in the first stage of surgery because untreated fistulas often show more complications.[3] On the other hand, if the entire colon is dilated as a pouch or is not of adequate length to accomplish pull-through, a coloplasty is required; tabularization of the pouch for about

15 cm length is essential to preserve the function of the colon, and then it should be brought out as an end colostomy followed by abdominoperineal pull-through of the tabularized colon later. There is a need for caution if the tabularized segments of the colon are longer than 15 cm, as these are more likely to be associated with stasis, dilation of the segment, and frequent complications in the post-pull-through period.

There is some hope as improved management has led to a reduction in mortality rates from 30–40% to 10–20%. We do have concerns about high fecal incontinence with rates as high as 60% in the initial years after the pull-through procedures, high urinary incontinence, especially in females with wide bladder neck and sacral deformities, and colonic re-dilatation due to altered histopathology in many cases. Our patient had anorectal agenesis, a short length of the colon, a pouch-like rectosigmoid, along with a genitourinary fistula, and no transition point between the pouch colon and normal colon. This fulfilled the criteria of CPC.[22] The colon did show an adequate length for a pull-through procedure, and hence it was an incomplete CPC. The blood supply could not be evaluated. Moreover, our patient had urethral hypoplasia, but with no sign of a posterior valve and no signs of urine retention, no specific action was required. After the definitive surgery, we kept a size 6 French urethral catheter in place for 7 days, and once it was removed, the urine output was adequate. Ultrasound also showed an acceptable normal urine residue. MCUG did not show hydroureteronephrosis or vesicoureteral reflux. As metabolic and genetic evaluation did not reveal a specific cause of the normal anion gap metabolic acidosis, we suspect it was due to chloride absorption in the urine that refluxed through the colovesical fistula to the colonic mucosa. This was supported by the fact that the acidosis had improved after the definitive surgery. The patient's right-sided ureterocele, along with right-sided ureterohydronephrosis, was followed up regularly after the definitive surgery, and these have resolved without intervention.

More recent recommendations suggest that the appendices should be removed at the time of pull-through to prevent misdiagnosis in the event of appendicitis occurring at a later date.[9] We did not do so in our case. On follow-up, our patient has shown normal neurodevelopment, tolerated oral intake, and is gaining weight appropriately. She is having recurrent UTI, which could be due to the previous fistulotomy. There have also been some episodes of diarrhea, which may be due to storage problems or fecal incontinence. We will know more once she gets to the age of urinary training.

Conclusion

Congenital pouch colon is a rare but important differential diagnosis of abdominal distention; it should be suspected in any case of ARM for timely diagnosis and management.

References

1. Chadha R. Congenital pouch colon associated with anorectal agenesis. Pediatr Surg Int 2004;20(6):393–401. DOI: 10.1007/s00383-004-1162-2.
2. Holschneider AM, Hutson JM. Anorectal Malformation in Children. Berlin: Springer-Verlag; 2006. p. 200.
3. Rao KL, Menon P. Congenital pouch colon associated with anorectal agenesis (pouch colon syndrome). Pediatr Surg Int 2005;21(2):125–126. DOI: 10.1007/s00383-004-1335-z.
4. Angotti R, Salih QQM, Molinaro F, et al. Congenital pouch colon associated with anorectal malformation: A rare anomaly of Asian Region—experience of Kurdish centre. Afr J Paediatr Surg 2018;15(1):10–15. DOI: 10.4103/ajps.AJPS_84_16.
5. Sangkhathat S, Patrapinyokul S, Chiengkriwate P. Functional and manometric outcomes after a congenital pouch colon reconstruction: Report of a case. J Med Assoc Thai 2012;95(2):270–274. PMID: 22435259.
6. Chadha R, Bagga D, Malhotra CJ, et al. The embryology and management of congenital pouch colon associated with anorectal agenesis. J Pediatr Surg 1994;29(3):439–446. DOI: 10.1016/0022-3468(94)90588-6.
7. Stephens FD, Smith ED, Paul NW. Anorectal Malformations in Children, 24. New York: Liss; 1988. pp. 105–110.
8. Wakhlu AK, Wakhlu A, Pandey A, et al. Congenital short colon. World J Surg 1996;20(1):107–114. DOI: 10.1007/s002689900019.
9. Gupta DK, Sharma S. Congenital pouch colon: Then and now. J Indian Assoc Pediatr Surg 2007;12(1):5–12. DOI: 10.4103/0971-9261.31081.
10. Alelayan AF, Ali W, Abdo A, et al. Congenital pouch colon associated with appendiceal duplication in two newborns. J Pediatr Surg Case Rep 2022;1(1):102124. DOI: 10.1016/j.epsc.2021.102124.
11. Spriggs N. Congenital occlusion of the gastrointestinal tract. Guys Hosp Rep 1912;766:143.
12. Narasimharao K. Congenital short colon with imperforate anus (pouch colon syndrome). Ann Paediatr Surg 1984;1(1):159–167. DOI: 10.5144/0256-4947.2007.79.
13. Saxena AK, Mathur P. Classification of congenital pouch colon based on anatomic morphology. Int J Colorectal Dis 2008;23(6):635–639. DOI: 10.1007/s00384-008-0450-z.
14. Nascimben F, Lehn A, Maldonado C, et al. Congenital pouch colon associated to a cloaca malformation in a syndromic newborn: A case report. J Pediatr Surg Case Rep 2023;99:102739. DOI: 10.1016/j.epsc.2023.102739.
15. Trusler GA, Mestel AL, Stephens CA. Colon malformation with imperforate anus. Surgery 1959;45(2):328–334. PMID: 13625011.
16. Gupta S, Mathur P, Mishra AK, et al. Whole exome-trio analysis reveals rare variants associated with congenital pouch colon. Children (Basel) 2023;10(5):902. DOI: 10.3390/children10050902.
17. Niaz S, Naz S, Raziq RA. Congenital pouch colon in a neonate. Pak J Med Sci 2022;38(2):426–429. DOI: 10.12669/pjms.38.ICON-2022.5771.
18. Maudar KK, Gandhi P, Varshney S, et al. Congenital pouch colon: Review of current clinical and molecular studies. J Pediatr Neonatal Care 2016;5(9):00227.
19. Shimotake T, Higuchi K, Tsuda T, et al. Infrared spectrophotometry of intraluminal meconium calculi in a neonate with imperforate anus and rectourethral fistula. J Pediatr Surg 2006;41(6):1173–1176. DOI: 10.1016/j.jpedsurg.2006.01.067.
20. Parelkar S, Oak S, Mishra PK, et al. Congenital pouch colon with rectal atresia: A case report. J Pediatr Surg 2010;45(3):639–641. DOI: 10.1016/j.jpedsurg.2009.12.028.
21. Mathur P, Prabhu K, Jindal D. Unusual presentations of pouch colon. J Pediatr Surg 2002;37(9):1351–1353. DOI: 10.1053/jpsu.2002.35007.
22. Sharma S, Gupta DK. Management options of congenital pouch colon: A rare variant of anorectal malformation. Pediatr Surg Int 2015;31(8):753–758. DOI: 10.1007/s00383-015-3739-3.

SHORT COMMUNICATION

Advances in Viscoelastic Coagulation Monitoring

Brunetta Guaragni

Received on: 21 September 2025; *Accepted on*: 29 November 2025; *Published on*: 15 January 2026

Abstract

Bleeding due to maturational and acquired coagulation disorders is seen frequently in premature/critically ill neonates. Viscoelastic coagulation monitoring (VCM) has been an exciting advance in the evaluation of blood clot formation, stabilization, and dissolution. Unlike the conventional evaluation of prothrombin time/international normalized ratio and activated partial thromboplastin time that are performed on plasma and do not provide information about platelets and fibrin cross-linking, viscoelastic tests (VETs) analyze whole blood and provide a global overview of the adequacy of clotting factors, fibrinogen, platelet function, red blood cells, and fibrinolytic processes. In the past few years, several cartridge-based devices have become available that do not need controlled pipetting. One VCM utilizes a miniature sensor without any moving mechanical parts to measure clot stiffness. It analyzes the coagulation state in a whole blood sample with a low-amplitude rotational or oscillatory force in less than 1 hour; it is portable, easy to use, and the small blood volume can be obtained from a heel stick. In this brief communication, the authors report VCM findings from a 1-day-old premature infant with hypofibrinogenemia and from a 5-day-old control of comparable gestational age. The altered parameters suggested suboptimal clot strength. These newer cartridge-based methods are being continuously improved; some instruments maintain the core VET principles of thromboelastography (TEG) and rotational thromboelastometry (ROTEM) but are being advanced with microfluidic cartridges and resonance-based detection instead of the rotating cup/pin of legacy devices, reducing sample handling and operator workload. Another ultrasound-based method measures the stiffness/shear modulus of whole blood as it clots. To reiterate, VETs can potentially improve hemostatic management, reduce unnecessary transfusions, and enhance patient outcomes. Further evaluation is definitely needed, but these tests could make a difference in the management of premature/critically ill infants.

Keywords: Amplitude, α-angle (rate of clot formation), Cartridge-based coagulation monitors, Clot formation time, Clot initiation, Clot kinetics, Clot strength, Clot strengthening, Clotting, Coagulation, Coagulation index, Clotting time, DIC, Estimated percentage of lysis, Fibrin activity, Fibrinolysis, Fibrin strands, Hypofibrinogenemia, Maximum clot strength, Maximum clot firmness, Premature, Shear modulus of whole blood, Thromboelastography, Thromboelastometry, Viscoelastic coagulation monitor.

Newborn (2025): 10.5005/jp-journals-11002-0139

Key Points

- Bleeding due to maturational and acquired coagulation disorders is seen frequently in premature and critically ill neonates.
- In these patients, standard coagulation laboratory tests might be unsuitable to investigate hemostatic function as these are affected by the concentrations of procoagulant but not anticoagulant proteins.
- Viscoelastic coagulation tests, by providing a comprehensive assessment of hemostasis, can help overcome some of these limitations.
- In this brief communication, we report findings using a viscoelastic coagulation monitor (VCM) from a 1-day-old premature infant with hypofibrinogenemia and a 5-day-old control of comparable gestational age to illustrate the potential utility of these assays.
- Newer cartridge-based methods are continuously becoming available. Some instruments use microfluidic cartridges and resonance-based detection. An ultrasound-based method that measures the stiffness/shear modulus of whole blood clots is being evaluated.

Advances in Viscoelastic Coagulation Monitoring

Viscoelastic coagulation monitoring (VCM) has been an exciting advance in the evaluation of blood clot formation, stabilization, and dissolution.[1–4] These tests analyze whole blood and provide a global overview of the adequacy of clotting factors, fibrinogen, platelet function, red blood cells, and fibrinolytic processes.[4,5] These represent a definite advancement over the conventional prothrombin time/international normalized ratio and activated partial thromboplastin time that are performed on plasma and do not provide information about platelets and fibrin cross-linking.[6–8] Viscoelastic tests (VETs) are also useful because these can be performed rapidly at the bedside and may optimize transfusion therapy for individualized management of at-risk infants.[9–11]

The past few years have seen major progress in VETs.[9] Several cartridge-based devices have become available that do not need controlled pipetting or the tube-pin systems in earlier thromboelastography (TEG) and rotational thromboelastometry (ROTEM) tests.[12] One of these is the VCM, which uses a miniature

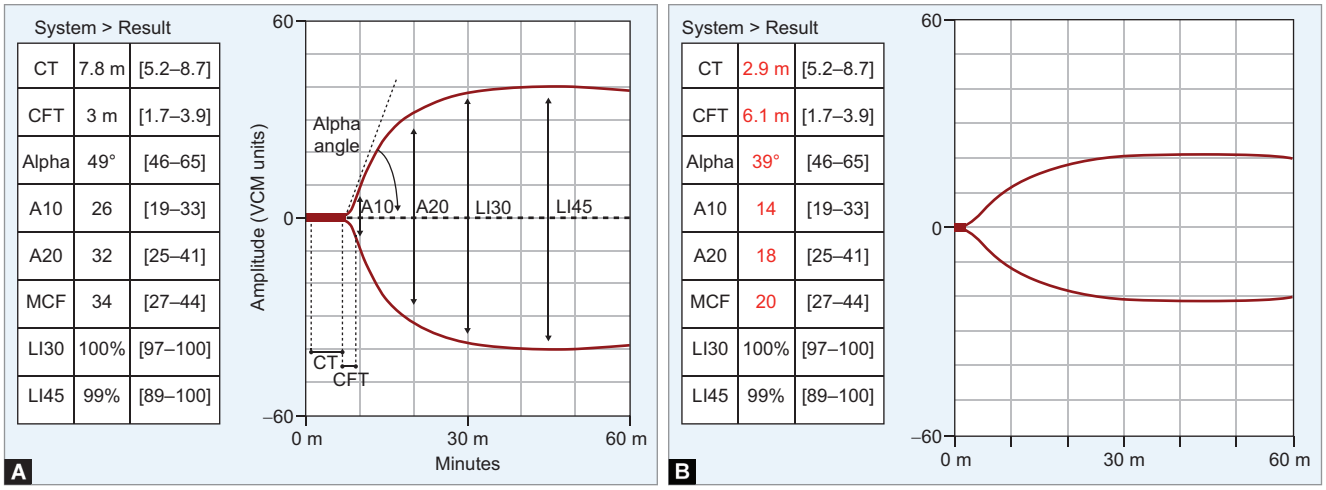
Department of Neonatology and Neonatal Intensive Care, Children's Hospital, ASST-Spedali Civili, Brescia, Italy; Global Newborn Society, Harrison, New York, United States of America

Corresponding Author: Brunetta Guaragni, Department of Neonatology and Neonatal Intensive Care, Children's Hospital, ASST-Spedali Civili, Brescia, Italy; Global Newborn Society, Harrison, New York, United States of America, e-mail: brunettaguaragni@gmail.com

How to cite this article: Guaragni B. Advances in Viscoelastic Coagulation Monitoring. Newborn 2025;4(4):225–227.

Source of support: Nil

Conflict of interest: None



Figs 1A and B: Viscoelastic coagulation monitoring in (A) A 5-day-old healthy preterm newborn (29^{+4} weeks of gestation); and (B) A 1-day-old preterm newborn (31 weeks of gestation) with hypofibrinogenemia (fibrinogen level was 0.7 grams/L)

CT, clotting time: time required to reach the clot amplitude of ≥1% above baseline; CFT, clot formation time: time required to pass from 1 to 10% amplitude of the clot signal, describing the kinetics of the clot; alpha angle: angle between the baseline and the tangent to the curve at an amplitude of 1%, describing the kinetics of the clot; A10: clot amplitude describing clot firmness at 10 minutes; A20: clot amplitude describing clot firmness at 20 minutes; MCF, maximum clot firmness: maximum amplitude reached before the beginning of fibrinolysis, describing firmness and quality of the clot; LI30, lysis at 30 minutes: percentage clot amplitude at 30 minutes referred to MCF; LI45, lysis at 45 minutes: percentage clot amplitude at 45 minutes referred to MCF

sensor without any moving mechanical parts to measure clot stiffness.[13,14] It requires a 300 µL fresh, whole blood sample and applies a low-amplitude rotational or oscillatory force to rapidly analyze the coagulation state in less than 1 hour; it is portable, easy to use, and the small blood volume can be obtained from a heel stick. As the blood begins to clot, the clot's viscoelastic resistance to that motion changes and sensors measure the changes in resistance and convert them into a clot-strength graph over time. The results are comparable to those obtained from standard blood samples.[15] Viscoelastic coagulation monitoring provides data about clot initiation (activity of coagulation pathways and formation of fibrin strands), clot kinetics (clot strengthening, indicating coagulation factor, and fibrin activity), maximum clot strength (contribution of fibrinogen and platelets), and fibrinolysis. In this brief communication, we report VCM findings from a 1-day-old premature infant with hypofibrinogenemia and from a 5-day-old control of comparable gestational age. The altered parameters suggest suboptimal clot strength[16,17] (highlighted in red font; Fig. 1).

As mentioned above, the best known comprehensive *in vitro* tests of coagulation prior to the availability of newer cartridge-based systems were TEG and ROTEM.[18,19] Thromboelastography is a VET method used to evaluate the entire coagulation process in real time.[16] A blood sample is placed in a gently oscillating tube that contains a pin and detects changes in resistance during clot formation. These mechanical changes are converted into a graphical trace that reflects key phases of coagulation, including clot initiation, rate of clot formation, maximum clot strength, and the degree of clot breakdown.[16,17,20] Rotational thromboelastometry measures the relative contribution of the intrinsic and extrinsic pathways, platelet function, fibrinogen levels, and fibrinolytic activity.[3] A blood sample is placed in a gently oscillating tube with a stationary pin, and an optical detection device measures changes in clot resistance.[3] There are several reagent-specific assays to test intrinsic activation (as in activated partial thromboplastin time test);[21,22] extrinsic activation (as in prothrombin time test);[19,22] fibrinogen assay,[9,11,15,19] which measures the contribution of fibrinogen in the absence of platelets; and the antifibrinolytic assay[9,11,15,19] that identifies excessive fibrinolysis.

Excitingly, there have been several recent advances even in the newer cartridge-based methods. Some of these newer instruments maintain the core VET principles of TEG and ROTEM but show modernized principles with microfluidic cartridges and resonance-based detection instead of the rotating cup/pin of legacy devices, reducing sample handling and operator workload.[23] Advanced ROTEM devices use freeze-dried reagents in cartridges and automatic sample handling to make the test faster, more standardized, and suitable for point-of-care applications.[24] Another device is ultrasound-based, and it measures the stiffness/shear modulus of whole blood as it clots.[25] The cartridge is fully sealed, has no moving mechanical parts in contact with the blood, and is more robust against vibration; these advances make it easier to operate, even outside a specialized lab environment.[26,27]

To reiterate, VETs can potentially improve hemostatic management, reduce unnecessary transfusions, and enhance patient outcomes.[20] Further evaluation is definitely needed, but these tests could make a difference in the management of premature/critically ill infants with multisystem organ failure.[28–30]

References

1. Cannata G, Mariotti Zani E, Argentiero A, et al. TEG((R)) and ROTEM((R)) traces: Clinical applications of viscoelastic coagulation monitoring in neonatal intensive care unit. Diagnostics (Basel) 2021;11(9):1642. DOI: 10.3390/diagnostics11091642.
2. Da Luz LT, Nascimento B, Shankarakutty AK, et al. Effect of thromboelastography (TEG(R)) and rotational thromboelastometry (ROTEM(R)) on diagnosis of coagulopathy, transfusion guidance and mortality in trauma: Descriptive systematic review. Crit Care 2014;18(5):518. DOI: 10.1186/s13054-014-0518-9.
3. Drotarova M, Zolkova J, Belakova KM, et al. Basic principles of rotational thromboelastometry (ROTEM((R))) and the role of ROTEM-guided fibrinogen replacement therapy in the management of

coagulopathies. Diagnostics (Basel) 2023;13(20):3219. DOI: 10.3390/diagnostics13203219.
4. Lawson PJ, Moore HB, Moore EE, et al. Preoperative thrombelastography maximum amplitude predicts massive transfusion in liver transplantation. J Surg Res 2017;220:171–175. DOI: 10.1016/j.jss.2017.05.115.
5. Carll T. Viscoelastic testing methods. Adv Clin Chem 2023;117:1–52. DOI: 10.1016/bs.acc.2023.09.001.
6. Dorgalaleh A, Favaloro EJ, Bahraini M, et al. Standardization of prothrombin time/international normalized ratio (PT/INR). Int J Lab Hematol 2021;43(1):21–28. DOI: 10.1111/ijlh.13349.
7. Ignjatovic V. Activated partial thromboplastin time. Methods Mol Biol 2013;992:111–120. DOI: 10.1007/978-1-62703-339-8_8.
8. Layibo Y, Padaro E, Magnang H, et al. Use of activated partial thrombin time and prothrombin time for quality assessment of fresh frozen plasma. Int J Hematol 2025;122(3):392–399. DOI: 10.1007/s12185-025-03984-4.
9. Amelio GS, Raffaeli G, Amodeo I, et al. Hemostatic evaluation with viscoelastic coagulation monitor: A NICU experience. Front Pediatr 2022;10:910646. DOI: 10.3389/fped.2022.910646.
10. Sewell EK, Forman KR, Wong EC, et al. Thromboelastography in term neonates: An alternative approach to evaluating coagulopathy. Arch Dis Child Fetal Neonatal Ed 2017;102(1):F79–F84. DOI: 10.1136/archdischild-2016-310545.
11. Parastatidou S, Sokou R, Tsantes AG, et al. The role of ROTEM variables based on clot elasticity and platelet component in predicting bleeding risk in thrombocytopenic critically ill neonates. Eur J Haematol 2021;106(2):175–183. DOI: 10.1111/ejh.13534.
12. Wells M, Raja M, Rahman S. Point-of-care viscoelastic testing. BJA Educ 2022;22(11):416–423. DOI: 10.1016/j.bjae.2022.07.003.
13. Roberts TR, Garcia I, Slychko I, et al. A deployable viscoelastic coagulation monitor enables point-of-care assessment of coagulopathy in swine with polytrauma. Mil Med 2025;190(5–6):e994–e1003. DOI: 10.1093/milmed/usae430.
14. Hennink I, Peters L, van Geest G, et al. Evaluation of a viscoelastic coagulation monitoring system (VCM Vet((R))) and its correlation with thromboelastometry (ROTEM((R))) in diseased and healthy dogs. Animals (Basel) 2023;13(3):405. DOI: 10.3390/ani13030405.
15. Schochl H, Forster L, Woidke R, et al. Use of rotation thromboelastometry (ROTEM) to achieve successful treatment of polytrauma with fibrinogen concentrate and prothrombin complex concentrate. Anaesthesia 2010;65(2):199–203. DOI: 10.1111/j.1365-2044.2009.06188.x.
16. Volod O, Runge A. Measurement of blood viscoelasticity using thromboelastography. Methods Mol Biol 2023;2663:709–724. DOI: 10.1007/978-1-0716-3175-1_47.
17. Willis J, Carroll C, Planz V, et al. Thromboelastography: A review for radiologists and implications on periprocedural bleeding risk. Abdom Radiol (NY) 2022;47(8):2697–2703. DOI: 10.1007/s00261-022-03539-9.
18. Korpallova B, Samos M, Bolek T, et al. Role of thromboelastography and rotational thromboelastometry in the management of cardiovascular diseases. Clin Appl Thromb Hemost 2018;24(8):1199–1207. DOI: 10.1177/1076029618790092.
19. Theusinger OM, Nurnberg J, Asmis LM, et al. Rotation thromboelastometry (ROTEM) stability and reproducibility over time. Eur J Cardiothorac Surg 2010;37(3):677–683. DOI: 10.1016/j.ejcts.2009.07.038.
20. Walsh M, Fritz S, Hake D, et al. Targeted thromboelastographic (TEG) blood component and pharmacologic hemostatic therapy in traumatic and acquired coagulopathy. Curr Drug Targets 2016;17(8):954–970. DOI: 10.2174/1389450117666160310153211.
21. Petricevic M, Biocina B, Milicic D, et al. Activated coagulation time vs intrinsically activated modified rotational thromboelastometry in assessment of hemostatic disturbances and blood loss after protamine administration in elective cardiac surgery: Analysis from the clinical trial (NCT01281397). J Cardiothorac Surg 2014;9:129. DOI: 10.1186/1749-8090-9-129.
22. Aires RB, Soares A, Gomides APM, et al. Thromboelastometry demonstrates endogenous coagulation activation in nonsevere and severe COVID-19 patients and has applicability as a decision algorithm for intervention. PLoS One 2022;17(1):e0262600. DOI: 10.1371/journal.pone.0262600.
23. Trevisan BM, Porada CD, Atala A, et al. Microfluidic devices for studying coagulation biology. Semin Cell Dev Biol 2021;112:1–7. DOI: 10.1016/j.semcdb.2020.06.002.
24. Faraoni D, DiNardo JA. Viscoelastic hemostatic assays: Update on technology and clinical applications. Am J Hematol 2021;96(10):1331–1337. DOI: 10.1002/ajh.26285.
25. Viola F, Mauldin FW Jr, Lin-Schmidt X, et al. A novel ultrasound-based method to evaluate hemostatic function of whole blood. Clin Chim Acta 2010;411(1–2):106–113. DOI: 10.1016/j.cca.2009.10.017.
26. Corey FS, Walker WF. Sonic estimation of elasticity via resonance: A new method of assessing hemostasis. Ann Biomed Eng 2016;44(5):1405–1424. DOI: 10.1007/s10439-015-1460-y.
27. Ferrante EA, Blasier KR, Givens TB, et al. A novel device for the evaluation of hemostatic function in critical care settings. Anesth Analg 2016;123(6):1372–1379. DOI: 10.1213/ANE.0000000000001413.
28. Dias JD, Levy JH, Tanaka KA, et al. Viscoelastic haemostatic assays to guide therapy in elective surgery: An updated systematic review and meta-analysis. Anaesthesia 2025;80(1):95–103. DOI: 10.1111/anae.16463.
29. Nair AB, Parker RI. Hemostatic testing in critically ill infants and children. Front Pediatr 2020;8:606643. DOI: 10.3389/fped.2020.606643.
30. Khizroeva J, Makatsariya A, Vorobev A, et al. The hemostatic system in newborns and the risk of neonatal thrombosis. Int J Mol Sci 2023;24(18):13864. DOI: 10.3390/ijms241813864.