October–December 2023 Volume 2 Issue 4 newborn Official Journal of the Global Newborn Society eISSN: 2769-514X

October–December 2023
Volume 2
Issue 4
eISSN: 2769-514X

newborn

Official Journal of the Global Newborn Society

December 3 is the International Day of Persons with Disability. The aim is to promote the rights and well-being of persons with disabilities in all spheres of society. A cardiologist mother has written about her own journey with her son who has grown with Trisomy 21, and about communication with families when they discover that their newborn infant has this condition. Contrary to popular belief, the journey has actually been rewarding.

IN A SPOT TO BREAK THAT NEWS?
Delivering a diagnosis of Down syndrome to an unaware family...

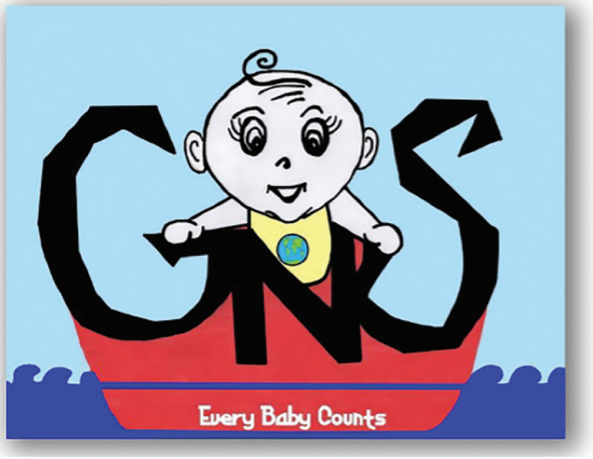
Other highlighted articles:

Bacteriophages

Organic acidemias: clinical presentation in neonates

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

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



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 2, Issue 4; October–December 2023

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

10.5005/newborn-2-4-iv

Early identification of birth defects can reduce secondary disabilities in newborn infants

Birth defects and developmental disabilities are important issues of public health concern.[1] Birth defects are abnormalities of structure, function, or metabolism that are present at birth and can result in physical or mental disability, or death.[2–5] About 3% of children born in the United States (US) have a major birth defect; these account for about 20% of all infant deaths.[4] Disability is defined as a limitation of activity associated with long-term physical, sensory, and/or cognitive impairments.[1,6] Developmental disabilities can begin *in utero* or after birth because of injury, infection, or other factors.[5] About 17% of children in the US have a developmental disability, with about 2% having a disability severe enough to require life-long care and special services.[1]

In 1992, the UN General Assembly proclaimed December 3rd as the International Day of Persons with Disabilities.[7–9] This day is celebrated every year to promote awareness and understanding of the problems faced by people with disabilities all over the world.[9] The goal is to promote inclusion, dignity, and the rights of affected people all over the world, be it in political, social, economic, and cultural life.[10] We need to be "United in action to rescue and achieve the Sustainable Development Goals (SDGs) for, with, and by persons with disabilities."[11,12] Unfortunately, much more work is still needed to achieve the SDGs by the original timeline of 2030.[13] The Children's Health Act of 2000 recognized the relevance of these conditions with the creation of the National Center on Birth Defects and Developmental Disabilities (NCBDDD) at the Centers for Disease Control and Prevention (CDC).[14,15] It has recently launched a national campaign, "Learn the Signs. Act Early," to educate parents and healthcare providers about the importance of early, timely intervention.[16–19]

We need a fundamental shift in our commitment, solidarity, and financing to reduce the public health burden emanating from these issues.[20] A political declaration at a recent SDG Summit[21] focused on the achievement of sustainable development and shared prosperity for all, by defining policies and actions that target the poorest and most vulnerable, including persons with disabilities.[22] The UN Disability Inclusion Strategy (UNSID; June 2019)[23] aims to raise the Organization's standards and performance on disability inclusion.[24,25] The idea was to provide sustainable and transformative progress in at-risk populations.[26]

Our journal, the *newborn* aims to cover fetal/neonatal problems that begin during pregnancy or occur after birth during the first 1000 days after birth. In this 4th issue of the second volume, we present 8 new articles (**Figure 1**). In neonates, Down syndrome is the most common genetic cause of intellectual disability; it affects approximately 1 in every 700 children, and accounts for around 15–20% of the intellectually disabled population.[27] The condition was first described by an English doctor, John Langdon Down,[28] and was subsequently associated with the trisomy of chromosome 21 by Professor Jerome Lejeune, a geneticist in Paris.[29,30] Since then, other less frequently seen forms of the condition have been discovered. Approximately, 94% of people with Down syndrome have standard trisomy 21, but 4% have a translocation and 2% have a mosaic Down syndrome.[31] In most cases, Down syndrome is not hereditary; it affects people of all ethnicities, religious backgrounds and economic situations.[32] It is covered under the Social Security Administration (SSA)'s "Blue Book" of impairments under Section 110.00.[33] In this issue, we bring a short communication from Dr Amita Garg, who is a renowned cardiologist in New Delhi, India, and is also a mother who has raised a child with trisomy 21.[34] She has first briefly shared her personal experience and then her views on how we medical professionals could/should interact with families in a positive and encouraging way. She has also listed resources that can help parents who are raising a child with this condition.

In another article, Singh *et al*.[35] have described the pathogenesis and implications of sensorineural hearing loss in children due to congenital CMV infections. These *in utero* infections account for nearly 25% of childhood hearing loss by the age of 4 years.[36] Hearing loss during childhood is an important disability with secondary effects on speech development and acquisition of linguistic skills.[37] A

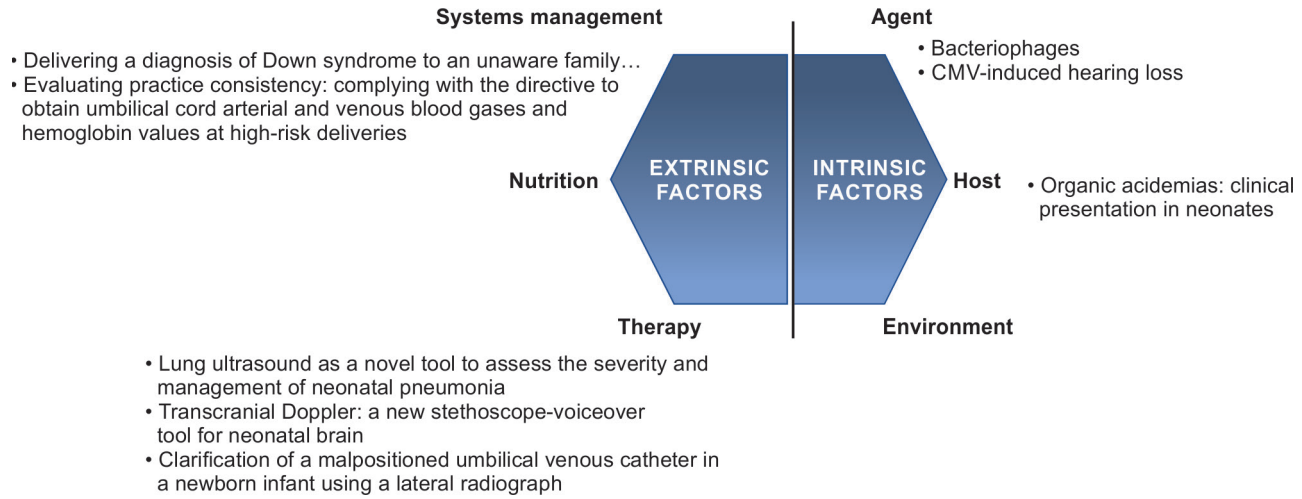


Fig. 1: **Areas of focus in the *newborn*, Volume 2, Issue 4.** We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health—those originating in therapy, nutrition, and systems management. This issue covers 4 of these foci, namely infectious diseases, host factors, treatment/monitoring systems, and systems management.

multidisciplinary approach is required; many children may need assistive hearing devices or cochlear implantation depending on the severity of their hearing loss.[38,39] In addition, early intervention services such as speech or occupational therapy may be needed.[38]

Kaushal and coworkers[40] have contributed with a detailed review of organic acidemias (OAs), which are heritable genomic abnormalities that result in accumulation of toxic organic acids. Most patients with severe deficiencies in the involved enzymatic pathways can become symptomatic in early infancy.[41,42] Acute clinical features include liver failure, lethargy, altered sensorium (encephalopathy), and/or seizures in the acute phase; subacute/delayed manifestations may include failure to thrive, developmental delay, and/or cardiomyopathy.[41,43,44] These manifestations can resemble those seen in systemic inflammatory response syndrome related to sepsis and a high index of suspicion is needed for timely diagnosis.[42]

In an interesting article, Tweddell and colleagues[45] report their experience with efforts to comply with the directive to obtain umbilical cord arterial and venous blood gases, and hemoglobin values at high-risk deliveries and then evaluate practice consistency.[46] They analyzed data from 1,050 births with placental abruption from a 24-month period. About 70% had both a cord arterial and venous gas, and hemoglobin levels reported. Acidosis was noted in 14%. In this subset, nearly 80% had abruption confirmed after birth. Fetal/neonatal anemia[47,48] was diagnosed in 12%. There is an opportunity to improve compliance with the directives to obtain cord arterial and venous blood gas and hemoglobin at high-risk births;[49] this may allow rapid evaluation of about 30% more high-risk infants for the presence of acidosis and anemia at birth.

Two articles emphasize the increasing importance of bedside sonography for monitoring critically ill infants. In the first, Kumar and Patodia[50] have reviewed cerebral Doppler ultrasound for tracking cerebral perfusion for monitoring critically ill very premature infants. They performed a comprehensive literature search using two databases and have provided an overview of several cerebral Doppler parameters such as the resistive index in the anterior cerebral artery in monitoring the evolution of neonatal disorders and/or response to therapy.[51–53] These data can also possibly help in predicting long-term neurodevelopment outcomes.[54] In another article, the same group has reviewed the importance of lung ultrasound as a tool for monitoring the severity of pneumonia in critically ill neonates in neonatal intensive care units. Durga *et al*.[55] have proposed a lung ultrasound (LUS) scoring system to help monitor the severity of pneumonia and its progression. Such bedside scores have been useful in adults with COVID-19 disease[56–58] and could help monitor the changing severity of lung disease in infants.

Singh *et al*.[59] have contributed another article focused on bacteriophages, viruses that invade bacterial cells.[60,61] Phages show a remarkable degree of diversity; recent advances in viral metagenomics show an unprecedented catalogue of phages in all microenvironments.[62] Phages may contain double-stranded (DS) DNA, single-stranded (SS) DNA, SS-RNA, and DS-RNA.[63] There is also a very high degree of structural diversity.[64] In terms of biological targets, these viruses attach and kill specific bacteria by expressing endolysins and holins without affecting the commensal microflora.[65,66] At the same time, bacteria are also developing numerous defense mechanisms to inhibit the phage life cycle.[67] Phages can inhibit some of these bacterial defenses, and the battle could go on.[68] These viruses may have translational importance with phage-based treatments; single phages, phage cocktails, phage-derived enzymes, phages in combination with antibiotics, and genetically modified phages might be useful in treating bacterial sepsis, even multidrug resistant (MDR) pathogens.[69,70]

Finally, Bottu and colleagues[71] submitted an interesting set of radiographs from a newborn infant in whom an umbilical arterial and an umbilical venous catheter (UVC) were inserted for stable vascular access. An anteroposterior radiograph showed the UVC was coiled up in the liver. Several possibilities including vascular abnormalities came to mind, but a lateral radiograph removed these doubts and was reassuring. The catheter was promptly removed, and the subsequent hospital stay of the infant was uneventful. They have reported these findings to re-emphasize that lateral radiographs can be useful.[72]

References

1. Boyle CA, Cordero JF. Birth defects and disabilities: a public health issue for the 21st century. Am J Public Health. 2005;95(11):1884–6. doi: 10.2105/AJPH.2005.067181.
2. Oliveira CI, Fett-Conte AC. Birth defects: Risk factors and consequences. J Pediatr Genet. 2013;2(2):85–90. doi: 10.3233/PGE-13052.
3. Malherbe HL, Modell B, Blencowe H, Strong KL, Aldous C. A review of key terminology and definitions used for birth defects globally. J Community Genet. 2023;14(3):241–62. doi: 10.1007/s12687-023-00642-2.
4. Wang Y, Hu J, Druschel CM. A retrospective cohort study of mortality among children with birth defects in New York State, 1983–2006. Birth Defects Res A Clin Mol Teratol. 2010;88(12):1023–31. doi: 10.1002/bdra.20711.
5. Prevention CfDCa. Data & Statistics on Birth Defects Atlanta: CDC; 2023 [Available from: https://www.cdc.gov/ncbddd/birthdefects/data.html.]
6. Babik I, Gardner ES. Factors Affecting the Perception of Disability: A Developmental Perspective. Front Psychol. 2021;12:702166. doi: 10.3389/fpsyg.2021.702166.
7. DeLisa JA. December 3—International Day of Persons with Disabilities: an opportunity to advocate for equal opportunities. Am J Phys Med Rehabil. 2012;91(11):999–1001. doi: 10.1097/PHM.0b013e31826edd7f.
8. Carmona RH, Giannini M, Bergmark B, Cabe J. The Surgeon General's Call to Action to Improve the Health and Wellness of Persons with Disabilities: historical review, rationale, and implications 5 years after publication. Disabil Health J. 2010;3(4):229–32. doi: 10.1016/j.dhjo.2010.07.004.
9. Northway R. Each December, the United Nations (UN) holds an 'International Day of Disabled Persons' to promote better understanding of disability. Introduction. J Intellect Disabil. 2013;17(4):281–2. doi: 10.1177/1744629513512248.
10. Nations U. Vision for an Inclusive Society New York: United Nations; 2009 [Available from: https://www.un.org/esa/socdev/documents/compilation-brochure.pdf.]
11. WORKERS IFOS. UNITED IN ACTION TO RESCUE AND ACHIEVE THE SDGS FOR, WITH AND BY PERSONS WITH DISABILITIES. Rheinfelden, Switzerland: International Federation of Social Workers; 2023 [Available from: https://www.ifsw.org/united-in-action-to-rescue-and-achieve-the-sdgs-for-with-and-by-persons-with-disabilities/.]

12. Development UDoEaSAS. The 17 Goals: United Nations; 2023 [Available from: https://sdgs.un.org/goals.]
13. Data OWi. SDG Tracker: Measuring progress towards the Sustainable Development Goals: Our World in Data team; 2023 [Available from: https://ourworldindata.org/sdgs.]
14. The National Children's Study Research Plan: A Review Washington (DC): National Academies Press (US); 2008 [Available from: https://www.ncbi.nlm.nih.gov/books/NBK20646/.]
15. Boyle CA, Cordero JF, Trevathan E. The National Center on Birth Defects and Developmental Disabilities: past, present, and future. Am J Prev Med. 2012;43(6):655–8. doi: 10.1016/j.amepre.2012.08.015.
16. Kretch KS, Willett SL, Hsu LY, Sargent BA, Harbourne RT, Dusing SC. "Learn the Signs. Act Early.": Updates and Implications for Physical Therapists. Pediatr Phys Ther. 2022;34(4):440–8. doi: 10.1097/PEP.0000000000000937.
17. Abercrombie J, Wiggins L, Green KK. CDC's "Learn the Signs. Act Early." Developmental Milestone Resources to Improve Early Identification of Children with Developmental Delays, Disorders, and Disabilities. Zero Three. 2022;43(1):5–12. PMCID: PMC10193264.
18. Gadomski AM, Riley MR, Scribani M, Tallman N. Impact of "Learn the Signs. Act Early." Materials on Parental Engagement and Doctor Interaction Regarding Child Development. J Dev Behav Pediatr. 2018;39(9):693–700. doi: 10.1097/DBP.0000000000000604.
19. Abercrombie J, Pann J, Shin F, Taylor E, Brisendine AE, Swanson-Holm R, et al. Evaluation of the Feasibility and Perceived Value of Integrating Learn the Signs. Act Early. Developmental Monitoring Resources in Early Head Start. Early Child Educ J. 2021;50. doi: 10.1007/s10643-021-01247-5.
20. Council GAEaS. Progress towards the Sustainable Development Goals: Towards a Rescue Plan for People and Planet New York: United Nations; 2023 [Available from: https://hlpf.un.org/sites/default/files/2023-04/SDG%20Progress%20Report%20Special%20Edition.pdf.]
21. Nations U. United Nations SDG Summit 2023 New York: United Nations; 2023 [Available from: https://www.un.org/en/conferences/SDGSummit2023/political-declaration.
22. Nations U. International Day of Persons with Disabilities, 3 December New York: United Nations; 2023 [Available from: https://www.un.org/en/observances/day-of-persons-with-disabilities.]
23. Nations U. United Nations Disability Inclusion Strategy New York: United Nations; 2019 [Available from: https://www.un.org/en/content/disabilitystrategy/.]
24. Stuart H. United Nations convention on the rights of persons with disabilities: a roadmap for change. Curr Opin Psychiatry. 2012;25(5):365–9. doi: 10.1097/YCO.0b013e328356b7ed.
25. McCusker P, Gillespie L, Davidson G, Vicary S, Stone K. The United Nations Convention on the Rights of Persons with Disabilities and Social Work: Evidence for Impact? Int J Environ Res Public Health. 2023;20(20). doi: 10.3390/ijerph20206927.
26. Nations U. The Sustainable Development Agenda New York: United Nations; 2023 [Available from: https://www.un.org/sustainabledevelopment/development-agenda/.]
27. Abukhaled Y, Hatab K, Awadhalla M, Hamdan H. Understanding the genetic mechanisms and cognitive impairments in Down syndrome: towards a holistic approach. J Neurol. 2023. doi: 10.1007/s00415-023-11890-0.
28. Van Robays J. John Langdon Down (1828–1896). Facts Views Vis Obgyn. 2016;8(2):131–6. PMCID: PMC5130304.
29. Megarbane A, Ravel A, Mircher C, Sturtz F, Grattau Y, Rethore MO, et al. The 50th anniversary of the discovery of trisomy 21: the past, present, and future of research and treatment of Down syndrome. Genet Med. 2009;11(9):611–6. doi: 10.1097/GIM.0b013e3181b2e34c.
30. Korenberg JR. Down syndrome: the crucible for treating genomic imbalance. Genet Med. 2009;11(9):617–9. doi: 10.1097/GIM.0b013e3181b765e7.
31. Halder P, Pal U, Ganguly A, Ghosh P, Ray A, Sarkar S, et al. Understanding etiology of chromosome 21 nondisjunction from gene X environment models. Sci Rep. 2021;11(1):22390. doi: 10.1038/s41598-021-01672-x.
32. Hertfordshire Uo. Intellectual Disability and Health: Down's syndrome. Hertfordshire, United Kingdom: University of Hertfordshire; 2023 [Available from: http://www.intellectualdisability.info/historic-articles/articles/downs-syndrome.]
33. Administration SS. Disability Evaluation Under Social Security. Baltimore, MD: Social Security Administration; 2023 [Available from: https://www.ssa.gov/disability/professionals/bluebook/.]
34. Garg A. Delivering a diagnosis of Down syndrome to an unaware family…. Newborn (Clarksville). 2023;2(4):245–8. doi: 10.5005/jp-journals-11002-0082.
35. Singh S, Maheshwari A, Bopanna S. CMV-induced hearing loss. Newborn (Clarksville). 2023;2(4):249–62. doi: 10.5005/jp-journals-11002-0081.
36. Fowler KB. Congenital cytomegalovirus infection: audiologic outcome. Clin Infect Dis. 2013;57 Suppl 4(Suppl 4):S182–4. doi: 10.1093/cid/cit609.
37. Cupples L, Ching TY, Crowe K, Seeto M, Leigh G, Street L, et al. Outcomes of 3-year-old children with hearing loss and different types of additional disabilities. J Deaf Stud Deaf Educ. 2014;19(1):20–39. doi: 10.1093/deafed/ent039.
38. Singh G, Gaidhane A. A Review of Sensorineural Hearing Loss in Congenital Cytomegalovirus Infection. Cureus. 2022;14(10):e30703. doi: 10.7759/cureus.30703.
39. Asghari A, Daneshi A, Farhadi M, Ajalloueyan M, Rajati M, Hashemi SB, et al. Complications and outcomes of cochlear implantation in children younger than 12 months: A multicenter study. Int J Pediatr Otorhinolaryngol. 2023;167:111495. doi: 10.1016/j.ijporl.2023.111495.
40. Kaushal M, Athalye-Jape G, Rahman MM, Motta M. Organic Acidemias: Clinical Presentation in Neonates. Newborn (Clarksville). 2023;2(4):263–78. doi: 10.5005/jp-journals-11002-0080.
41. Shennar HK, Al-Asmar D, Kaddoura A, Al-Fahoum S. Diagnosis and clinical features of organic acidemias: A hospital-based study in a single center in Damascus, Syria. Qatar Med J. 2015;2015(1):9. doi: 10.5339/qmj.2015.9.
42. Ramsay J, Morton J, Norris M, Kanungo S. Organic acid disorders. Ann Transl Med. 2018;6(24):472. doi: 10.21037/atm.2018.12.39.
43. Park KC, Krywawych S, Richard E, Desviat LR, Swietach P. Cardiac Complications of Propionic and Other Inherited Organic Acidemias. Front Cardiovasc Med. 2020;7:617451. doi: 10.3389/fcvm.2020.617451.
44. Chen B, Zhan Y, Kessi M, Chen S, Xiong J, Deng X, et al. Urine organic acids as metabolic indicators for global developmental delay/intellectual disability in Chinese children. Front Mol Biosci. 2021;8:792319. doi: 10.3389/fmolb.2021.792319.
45. Tweddell SM, Bahr TM, Ohls RK, Christensen RD. Evaluating practice consistency: complying with the directive to obtain umbilical cord arterial and venous blood gasses, and hemoglobin values, at high-risk deliveries. Newborn (Clarksville). 2023;2(4):310–3. doi: 10.5005/jp-journals-11002-0075.

46. Practice ACoO. ACOG Committee Opinion No. 348, November 2006: Umbilical cord blood gas and acid-base analysis. Obstet Gynecol. 2006; 108(5):1319–22. doi: 10.1097/00006250-200611000-00058.
47. Nassin ML, Lapping-Carr G, de Jong JL. Anemia in the Neonate: The Differential Diagnosis and Treatment. Pediatr Ann. 2015;44(7):e159-63. doi: 10.3928/00904481-20150710-08.
48. Christensen RD. A Guide to Identifying the Cause of Anemia in a Neonate. In: de Alarcón PA, Werner EJ, Christensen RD, Sola-Visner MC, editors. Neonatal Hematology: Pathogenesis, Diagnosis, and Management of Hematologic Problems. 3rd ed. Cambridge, United Kingdom: Cambridge University Press; 2021. p. iv.
49. Saneh H, Mendez MD, Srinivasan VN. Cord Blood Gas Treasure Island (FL): StatPearls Publishing; 2023 [Available from: https://www.ncbi.nlm.nih.gov/books/NBK545290/.]
50. Kumar G, Patodia J, Padhan N, Maheshwari A, Suryawanshi P. Transcranial Doppler: A New Stethoscope- Voiceover Tool for Neonatal brain. Newborn (Clarksville). 2023;2(4):279–90. doi: 10.5005/jp-journals-11002-0077.
51. Snyder EJ, Perin J, Chavez-Valdez R, Northington FJ, Lee JK, Tekes A. Head ultrasound resistive indices are associated with brain injury on diffusion tensor imaging magnetic resonance imaging in neonates with hypoxic-ischemic encephalopathy. J Comput Assist Tomogr. 2020;44(5):687–91. doi: 10.1097/RCT.0000000000001069.
52. Ecury-Goossen GM, Raets MM, Camfferman FA, Vos RH, van Rosmalen J, Reiss IK, et al. Resistive indices of cerebral arteries in very preterm infants: values throughout stay in the neonatal intensive care unit and impact of patent ductus arteriosus. Pediatr Radiol. 2016;46(9):1291–300. doi: 10.1007/s00247-016-3615-x.
53. Akin MS, Sari FN, Ceran B, Bozkaya D, Okman E, Alkan M, et al. Cerebral monitoring of very preterm infants with anterior cerebral artery resistive index and early NIRS. Turk J Med Sci. 2023;53(1):225–32. doi: 10.55730/1300-0144.5577.
54. Camfferman FA, de Goederen R, Govaert P, Dudink J, van Bel F, Pellicer A, et al. Diagnostic and predictive value of Doppler ultrasound for evaluation of the brain circulation in preterm infants: a systematic review. Pediatr Res. 2020;87(Suppl 1):50–8. doi: 10.1038/s41390-020-0777-x.
55. Durga D, Devi U, Gupta K, Maheshwari A, Suryawanshi P. Lung ultrasound as a novel tool to assess the severity and management of neonatal pneumonia. Newborn (Clarksville). 2023;2(4):291–6. doi: 10.5005/jp-journals-11002-0076.
56. Dell'Aquila P, Raimondo P, Racanelli V, De Luca P, De Matteis S, Pistone A, et al. Integrated lung ultrasound score for early clinical decision-making in patients with COVID-19: results and implications. Ultrasound J. 2022;14(1):21. doi: 10.1186/s13089-022-00264-8.
57. Zhu F, Zhao X, Wang T, Wang Z, Guo F, Xue H, et al. Ultrasonic characteristics and severity assessment of lung ultrasound in COVID-19 pneumonia in Wuhan, China: A retrospective, observational study. Engineering (Beijing). 2021;7(3):367–75. doi: 10.1016/j.eng.2020.09.007.
58. Dargent A, Chatelain E, Kreitmann L, Quenot JP, Cour M, Argaud L, et al. Lung ultrasound score to monitor COVID-19 pneumonia progression in patients with ARDS. PLoS One. 2020;15(7):e0236312. doi: 10.1371/journal.pone.0236312.
59. Singh S, Nath G, Maheshwari A. Bacteriophages. Newborn (Clarksville). 2023;2(4):297–309. doi: 10.5005/jp-journals-11002-0078.
60. Clokie MR, Millard AD, Letarov AV, Heaphy S. Phages in nature. Bacteriophage. 2011;1(1):31–45. doi: 10.4161/bact.1.1.14942.
61. Kasman LM, Porter LD. Bacteriophages. StatPearls. Treasure Island (FL) 2023.
62. Dion MB, Oechslin F, Moineau S. Phage diversity, genomics and phylogeny. Nat Rev Microbiol. 2020;18(3):125–38. doi: 10.1038/s41579-019-0311-5.
63. Nguyen HM, Watanabe S, Sharmin S, Kawaguchi T, Tan XE, Wannigama DL, et al. RNA and single-stranded DNA phages: Unveiling the promise from the underexplored world of viruses. Int J Mol Sci. 2023;24(23). doi: 10.3390/ijms242317029.
64. Somerville V, Schowing T, Chabas H, Schmidt RS, von Ah U, Bruggmann R, et al. Extensive diversity and rapid turnover of phage defense repertoires in cheese-associated bacterial communities. Microbiome. 2022;10(1):137. doi: 10.1186/s40168-022-01328-6.
65. Oliveira H, Sao-Jose C, Azeredo J. Phage-Derived Peptidoglycan Degrading Enzymes: Challenges and Future Prospects for In Vivo Therapy. Viruses. 2018;10(6). doi: 10.3390/v10060292.
66. Roach DR, Donovan DM. Antimicrobial bacteriophage-derived proteins and therapeutic applications. Bacteriophage. 2015;5(3):e1062590. doi: 10.1080/21597081.2015.1062590.
67. Seed KD. Battling Phages: How bacteria defend against viral attack. PLoS Pathog. 2015;11(6):e1004847. doi: 10.1371/journal.ppat.1004847.
68. Yirmiya E, Leavitt A, Lu A, Ragucci AE, Avraham C, Osterman I, et al. Phages overcome bacterial immunity via diverse anti-defence proteins. Nature. 2023. doi: 10.1038/s41586-023-06869-w.
69. Petrovic Fabijan A, Iredell J, Danis-Wlodarczyk K, Kebriaei R, Abedon ST. Translating phage therapy into the clinic: Recent accomplishments but continuing challenges. PLoS Biol. 2023;21(5):e3002119. doi: 10.1371/journal.pbio.3002119.
70. Ling H, Lou X, Luo Q, He Z, Sun M, Sun J. Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era. Acta Pharm Sin B. 2022;12(12):4348–64. doi: 10.1016/j.apsb.2022.05.007.
71. Bottu A, O'Neal L, Manzar S. Identifying malposition of umbilical venous catheter using a lateral film. Newborn (Clarksville). 2023;2(4):314–5. doi: 10.5005/jp-journals-11002-0079.
72. Butler GC, Al-Assaf N, Tarrant A, Ryan S, El-Khuffash A. Using lateral radiographs to determine umbilical venous catheter tip position in neonates. Ir Med J. 2014;107(8):256–8. PMID: 25282975.

Akhil Maheshwari, MD
Kei Lui, MD
Mario Motta, MD

SHORT COMMUNICATION

In a Spot to Break that News? Delivering a Diagnosis of Down Syndrome to an Unaware Family

Amita Garg

Received on: 06 November 2023; *Accepted on*: 12 December 2023; *Published on*: 05 January 2024

Abstract

This communication presents the views of a cardiologist mother who is raising a child with Down Syndrome/Trisomy 21. She has described her initial emotional struggles and then the subsequent journey, which has required her best efforts to support the child. In this article, she has focused on how medical professionals can make an impact by interacting with families in a positive, encouraging way, and has listed resources that may be of help. She reminds us that there is considerable variability in the outcomes of these children, and hence, not to unnecessarily begin with a negative view of the prognosis. It is important for us to not give up; society must come together, and courageous efforts can help optimize the care for these children and families.

Keywords: Alpha fetoprotein, Atrial septal defect, AV canal defects, Babies with Down Syndrome, Beta-human chorionic gonadotropin, Delivering the diagnosis of Down Syndrome, Dimeric inhibin A, Down Syndrome Education International, Down Syndrome Federation of India, Fetal echocardiography, John Langdon Down, Level 2 ultrasonography, Mongoloid, National Down Syndrome Society, Patent ductus arteriosus, Penta screen, Prenatal counseling, Trisomy 21, Unconjugated estriol, Ventricular septal defect.

Newborn (2023): 10.5005/jp-journals-11002-0082

Key Points

- Counseling a family with a fetus/newborn with Trisomy 21 can be difficult.
- It is important to carefully choose words when informing families about their fetus/infant having Trisomy 21.
- The management of a child with Trisomy 21 becomes easier in most cases after the first 2 years, and so an optimistic outlook is well-justified.
- There are several well-established medical/social resources that can be helpful.

Non-invasive Cardiology, Fortis Hospital, New Delhi, India

Corresponding Author: Amita Garg, Non-invasive Cardiology, Fortis Hospital, New Delhi, India, Phone: +91 9312239463, e-mail: singhal.amita@gmail.com

How to cite this article: Garg A. In a Spot to Break that News? Delivering a Diagnosis of Down Syndrome to an Unaware Family. Newborn 2023;2(4):245–248.

Source of support: Nil

Conflict of interest: None

Counseling Parents about Having a Fetus/Newborn Infant with Down Syndrome

Clinicians in newborn units often find themselves in a spot where they have to break the news to a family that their newborn infant may have Down Syndrome. It isn't easy and there isn't a perfect way to do it.

Being a mom to a child with Trisomy 21 and a medical professional at the same time puts me in a relatively unique position from where I can feel the joys and apprehensions, fears and elations of a parent. But I also need to look at the various aspects from a factual medical perspective.

Down, not Down's.[1–3] Down Syndrome is named after the English physician John Langdon Down,[4] who characterized the condition; he himself was not affected by it. An apostrophe with "s" connotes ownership or possession.[3] Personally, I prefer the nomenclature Trisomy 21 – it is more neutral and does away with the insinuating implications of the word "syndrome." Why a bias to start with?

As an active member of a Down Syndrome parent support group, I have had the privilege of interacting with many parents of kids with Trisomy 21. Having myself traversed this journey, I can feel the desperation of these new parents to hear some hopeful words. At the same time, I can also see my colleague's apprehensions of giving false hopes to the parents about the future of their child.

So when I was asked to write for this esteemed journal, I decided to take this opportunity to bring forth a parent's perspective who has been raising a child with Trisomy 21 for over a decade and who also understands the dilemma of a pediatrician/neonatologist/radiologist/obstetrician/cardiologist who at times during their career are put in a spot to "Break the News" of a child having Trisomy 21 to her/his parents and family.

Hope this helps.

My Own Journey

I will first share my own story…

During my second pregnancy, my Penta screen (α-fetoprotein, β-human chorionic gonadotropin, unconjugated estriol, dimeric inhibin A), fetal echocardiography, and a level 2 ultrasonography missed a ventricular septal defect, an atrial septal defect and all other sono-morphological features of Trisomy 21 itself !

Fig. 1: My son with Trisomy 21 (center, identification card with red borders). He is doing well emotionally, socially, and academically. We should never prematurely give up on our expectations of the future of a child, and therefore, the choice of words for delivering the information of this diagnosis to an unaware family is important. The possibilities are enormous and are continuously improving

In retrospective I would say I was fortunate...

Why? Because all of us know about the likely outcome of this pregnancy had we been informed about the diagnosis of Trisomy 21 prior to birth. Nonetheless, there are instances where parents have decided to give birth to fetuses and even twins with Trisomy 21.

As medical professionals, we are mostly involved with the diagnosis and management of surgical or medical issues. It might only be a brief snapshot of these patients' lives. We might not be able to see how their life shapes up; the beautiful, shared moments that the future holds, and the potential that these infants carry despite all difficulties. The first few years are difficult, but life may not be so bad later. Due to a lack of information and the *almost certain* uncertainty of bringing these kids to life, our algorithm has only one answer to pregnancies with chromosomal abnormalities—medical termination.

And that's precisely why I am writing this post.

When I got the diagnosis of Trisomy 21 in my newborn, it was hard to believe. At the outset, there was the natural non-acceptance – denial – questioning (why me?) – and heart-wrenching devastation, in that order. Mostly because...

I knew very little about what lay ahead. I was given a huge responsibility which I wasn't prepared for. And that was all about it.

I wish I had an instruction manual back then.

But once that initial trauma and shock wore off, things started to fall into place. My son began reading at 2 years of age (way before many of his neurotypical peers), following a mainstream syllabus, learning to skate, and riding a bicycle. He goes to one of the finest mainstream schools, the same educational center that accepted his older "scholar" sister. He is his teachers' pride, a favorite amongst friends and neighbors (Fig. 1). And above all, he is the greatest stress-buster of the house. I can easily say he is the only one in the house who is able to take away everyone's stress, without absorbing any himself!

It would be dishonest to say that raising a child with different needs, is a cakewalk. It never was and it will never be so. But there are rewards. Enormous.

And this is not just my story. Anyone who has happened to be around anyone with Trisomy 21 resonates with what I am saying. In today's era, people with Trisomy 21 are proving to be good students, sports personnel, professionals, and most importantly, wonderful human beings. Thanks to my son, I have been very fortunate to have met many amazing pediatricians and wonderful people. I sincerely hope and pray that every family blessed with a kid with Trisomy 21 finds comfort and hope in their pediatrician and in the larger society.

My Humble Suggestions

This is what I have learned. My most humble request to my medical colleagues—whosoever lands in a position to deliver a diagnosis of Trisomy 21 to a family:

1. Please always remember that the parent "IS NOT" prepared to hear what you are going to say!
   a. Please be very sensitive—this is going to change their lives forever.
   b. Words should be chosen carefully. Using the right words at the outset is important; these will make a lasting impact.
2. Parents must receive the news together.
   a. Please ensure they are holding the baby when they get the news and address the baby with her/his name if it has been decided.
   b. "Baby has Trisomy 21" is a better way, than to say that it's a Down Syndrome baby.
   c. The word "Mongoloid" should never be used. It is also legally prohibited.[5,6]
   d. A good way to begin is by congratulating them for their new baby and not with "I am sorry." Consistency is important; the attending pediatrician and obstetrician should convey the same message. Other staff members should preferably leave the discussions to the clinical leaders and not share their personal opinions with the family.
3. Every city across the globe has existing parent support groups for Trisomy 21. It will be a great idea to have an experienced parent on board while informing/counseling the parents. Please try.[7]

   At that point of time parents will connect with other parents better than the professionals.[7]

4. Please tell them that it is not a catastrophe to have a baby with Trisomy 21.

   It is OK and it is doable.[8]

5. Having a baby with Trisomy 21 has nothing to do with any of the parent's wrongdoing/karma/previous life. THIS IS NATURE. Statistically, every 700–1000th child will have one extra chromosome and this child just happens to be that 1000th child, that's it.[8]
6. This is the most frequently seen and most manageable congenital condition.[9] This is NOT a disease!
7. There might be health issues related to cardiac/endocrine/hearing and visual function. However, these are more difficult during the first 2 years, and subsequently, the health is more likely to follow a better track.[9,10]
   a. In the absence of compelling reasons, the family should be sent home early.[10] They'd need some time together.
   b. Parents should be encouraged to come for follow-up visits to screen for possible medical concerns.[10]
8. Many health issues, such as cardiac lesions can be permanently dealt with, in one go.[11] Others are more easily correctable during follow-up visits.

Fig. 2: These two photographs again emphasize that the possibilities are enormous. I personally cannot do many things that he can!

9. Physical milestones and intellectual delays are expected[12] but a lot can be done about these (Fig. 2).
10. Lifespan can be near normal in today's era of ever-improving medical facilities.[13]
11. There is no huge financial liability that comes with this condition; most issues can be handled with "common-sense parenting."[14] Having a child with Trisomy 21 does not automatically mean that there will be a permanent need for the parents to see doctors and therapists all their lives.

    Parents are the best, most effective, least expensive, and most easily accessible therapists!

12. "Better qualified and financially sound families living in bigger cities can do a better job raising kids with Trisomy 21" – *is a myth*.
    a. Common sense has nothing to do with qualification, wealth, or facilities available in metropolitan areas.
    b. I would really like to emphasize this. All training protocols work best and are most effective when done at home, by the family, and in the natural environment of the child.

    Families with most testing conditions have often shown the best results!

13. Having a child with Trisomy 21 DOES NOT necessarily mean that either/both parents would have to sacrifice their professional growth. Please relax! Having a child with this diagnosis does not automatically imply this; most setbacks are likely to be temporary. The initial years will demand more care and time,[15] a little more than a typical newborn would in catching up with the milestones and language development.
    a. The special needs gradually improve in most children. If both parents and the extended family decide to work in harmony, it will work wonders for the kid and also enhance the bonding among the family members. They will mostly be able to reorganize their schedules, manage the situation, and realize their professional dreams too. But, for this to happen, we as professionals need to hold hands and remove the negative misconceptions from new parent's minds.
    b. *Soon enough, the parents will know more about Trisomy 21 than professionals! All of us, need to regularly update ourselves, especially before we are to counsel a new family.*[16] Moralistic professionalism.
14. We need not always be too factual in one go. The flow of information can be gradual, or swift, per the needs of the situation.
15. Guide them on what to google. I have found many authentic websites, books, and resources. A few of these are:
    a. #NDSS (National Down Syndrome society);[17]
    b. #Down Syndrome Education International;[18]
    c. #Down Syndrome Federation of India;[19] and
    d. #Babies with Down Syndrome "A new parents guide" book/e-book.[20]
    e. There are many other books dedicated to each developmental area: gross and fine motor skills, language, nutrition, adolescence, just to name a few. Google can be useful – a googol number of possibilities!
16. We live in an era of ever-improving medical facilities and freely-available, evidence-based knowledge. Thankfully, the internet has democratized the access to information and knowledge. It has the potential to keep parents abreast with the latest global advances in management.
    a. Individuals with Trisomy 21 have shown improvements in cognitive function and independence over time.[21]
    b. Appropriate and timely intellectual stimulation helps.

Undoubtedly, it would require hard work, dedication and patience from the family. But medical professionals have bigger shoes to fill as besides providing necessary medical care we need to give them a sympathetic ear and wise counsel, hold their hands in times of distress and motivate them to help the individuals with Trisomy 21 fulfil their destiny. Let's spread hope together and avoid being remembered by the family as a pessimistic clinician.

Please try not to predict the future of the child, for the possibilities of growth are enormous.

NEVER GIVE UP ON ANY CHILD! Every baby counts!

References

1. Ward OC. John Langdon Down: The man and the message. Downs Syndr Res Pract 1999;6(1):19–24. DOI: 10.3104/perspectives.94.
2. Antonarakis SE, Skotko BG, Rafii MS, et al. Down syndrome. Nat Rev Dis Primers 2020;6(1):9. DOI: 10.1038/s41572-019-0143-7.
3. Society NDS. Preferred Language Washington DC: NDSS; 2023. Available from: https://ndss.org/preferred-language.
4. Birch CA. Down's syndrome. John Langdon Haydon Langdon-Down 1828–1896. Practitioner 1973;210(255):171–172. PMID: 4268293.
5. Rodriguez-Hernandez ML, Montoya E. Fifty years of evolution of the term Down's syndrome. Lancet 2011;378(9789):402. DOI: 10.1016/S0140-6736(11)61212-9.
6. Pennsylvania GAotCo. Down's syndrome – Prohibiting use of term "Mongolism". Act of Oct. 10, 1980, P.L. 799, No. 149 1980. Available from: https://www.legis.state.pa.us/WU01/LI/LI/US/PDF/1980/0/0149..PDF.
7. Society NDS. Friendships & Social Relationships Washington, DC: National Down Syndrome Society; 2023. Available from: https://ndss.org/resources/friendships-social-relationships.
8. Sheets KB, Crissman BG, Feist CD, et al. Practice guidelines for communicating a prenatal or postnatal diagnosis of Down

syndrome: Recommendations of the national society of genetic counselors. J Genet Couns 2011;20(5):432–441. DOI: 10.1007/s10897-011-9375-8.
9. National Center on Birth Defects and Developmental Disabilities CfDCaP. Data and Statistics on Down Syndrome Altanta, GA: Centers for Disease Control and Prevention; 2023. Available from: https://www.cdc.gov/ncbddd/birthdefects/downsyndrome/data.html.
10. Tenenbaum A, Hanna RN, Averbuch D, et al. Hospitalization of children with Down syndrome. Front Public Health 2014;2:22. DOI: 10.3389/fpubh.2014.00022.
11. Dimopoulos K, Constantine A, Clift P, et al. Cardiovascular complications of Down syndrome: Scoping review and expert consensus. Circulation 2023;147(5):425–441. DOI: 10.1161/CIRCULATIONAHA.122.059706.
12. Baksh RA, Pape SE, Chan LF, et al. Multiple morbidity across the lifespan in people with Down syndrome or intellectual disabilities: A population-based cohort study using electronic health records. Lancet Public Health 2023;8(6):e453–e462. DOI: 10.1016/S2468-2667(23)00057-9.
13. Esbensen AJ. Health conditions associated with aging and end of life of adults with Down syndrome. Int Rev Res Ment Retard 2010;39(C):107–126. DOI: 10.1016/S0074-7750(10)39004-5.
14. Mason WA, Fleming CB, Ringle JL, et al. Reducing risks for problem behaviors during the high school transition: Proximal outcomes in the common sense parenting trial. J Child Fam Stud 2015;24(9):2568–2578. DOI: 10.1007/s10826-014-0059-5.
15. Huiracocha L, Almeida C, Huiracocha K, et al. Parenting children with Down syndrome: Societal influences. J Child Health Care 2017;21(4):488–497. DOI: 10.1177/1367493517727131.
16. Jackson L, Cichon M, Kleinert H, et al. Teaching medical students how to deliver diagnoses of Down syndrome: Utility of an educational tool. Patient Educ Couns 2020;103(3):617–625. DOI: 10.1016/j.pec.2019.10.011.
17. Society NDS. Updates Washington, DC: National Down Syndrome Society; 2023. Available from: https://ndss.org/.
18. International DsE. Down syndrome Education International Newport Beach, CA: Down Syndrome Education International 2023. Available from: https://www.down-syndrome.org/en-us/.
19. India Dsfo. Down syndrome federation of India Chennai, India: Down syndrome federation of India; 2023. Available from: https://downsyndrome.in/.
20. Skallerup S. Babies with Down syndrome: A new parents' guide. 3rd ed. North Bethesda, Maryland: Woodbine House; 2008 09/28/2008,. p. 358.
21. Hamburg S, Lowe B, Startin CM, et al. Assessing general cognitive and adaptive abilities in adults with Down syndrome: A systematic review. J Neurodev Disord 2019;11(1):20. DOI: 10.1186/s11689-019-9279-8.

REVIEW ARTICLE

CMV-induced Hearing Loss

Srijan Singh[1,2], Akhil Maheshwari[2,3], Suresh Boppana[4]

Received on: 30 November 2023; *Accepted on*: 27 December 2023; *Published on*: 05 January 2024

Abstract

Congenital cytomegalovirus (cCMV) infection is the most common fetal viral infection and contributes to about 25% of childhood hearing loss by the age of 4 years. It is the leading nongenetic cause of sensorineural hearing loss (SNHL). Infants born to seroimmune mothers are not completely protected from SNHL, although the severity of their hearing loss may be milder than that seen in those whose mothers had a primary infection. Both direct cytopathic effects and localized inflammatory responses contribute to the pathogenesis of cytomegalovirus (CMV)-induced hearing loss. Hearing loss may be delayed onset, progressive or fluctuating in nature, and therefore, a significant proportion will be missed by universal newborn hearing screening (NHS) and warrants close monitoring of hearing function at least until 5–6 years of age. A multidisciplinary approach is required for the management of hearing loss. These children may need assistive hearing devices or cochlear implantation depending on the severity of their hearing loss. In addition, early intervention services such as speech or occupational therapy could help better communication, language, and social skill outcomes. Preventive measures to decrease intrauterine CMV transmission that have been evaluated include personal protective measures, passive immunoprophylaxis and valacyclovir treatment during pregnancy in mothers with primary CMV infection. Several vaccine candidates are currently in testing and one candidate vaccine in phase 3 trials. Until a CMV vaccine becomes available, behavioral and educational interventions may be the most effective strategy to prevent maternal CMV infection.

Keywords: ABR thresholds, Auditory brainstem response and otoacoustic emissions, Aural preference syndrome, Behavioral audiometry, *Betaherpesvirus*, Blood-labyrinth barrier, Cerebellar hypoplasia, Cerebral atrophy, CMV PCR, CMV-specific hyperimmune globulin, Cochlear blood–labyrinth barrier, Cochlear implant, Cytomegalic inclusion disease, Cytomegalovirus (CMV), Dried blood spot (DBS), Endocochlear potential, Fluctuating hearing loss, Ganciclovir, *Herpesviridae*, Human Herpes Virus 5, Icosahedral capsid, Impedance audiometry, Intracranial calcifications, Lenticulostriate vasculopathy, MF59-adjuvanted CMV glycoprotein B subunit vaccine , Migrational abnormalities, Natural killer, Neurotrophins, Newborn hearing screening, Nlrp3, Non-primary maternal infection, Organ of corti, Periventricular echo density, Play audiometry, Pure tone audiometry, Sensorineural hearing loss (SNHL), Seroimmune, Speech audiometry, Spiral ganglion cells, Spiral ganglion cells, Spiral ganglion neurons, Strain-specific epitopes, *Stria vascularis*, Tegument layer, Tympanometry, Unique long gene region, Unique short gene region, Valganciclovir, Ventricular adhesions, Ventricular dilatation, Ventriculomegaly, Viral core, Viral lipid bilayer envelope, Viral matrix, Viral replication cycle, Visual reinforcement audiometry, White matter disease.

Newborn (2023): 10.5005/jp-journals-11002-0081

Introduction

Congenital cytomegalovirus (cCMV) infection is the most common congenital infection with a birth prevalence reported around 0.64%.[1,2] Cytomegaloviruses are ubiquitous and the largest human viral pathogens with respect to genome size.[3–5] Morton and Nance estimated that cCMV contributes to 21% of all hearing loss at birth and 25% of childhood hearing loss by 4 years of age.[6] It is also a major cause of cognitive and neurologic deficits.[7]

Viral Structure

Cytomegalovirus (CMV), also known as human herpes virus 5, is classified in the *Herpesviridae* family and based on its ability to infect leukocytes, as a *beta-herpes virus*.[3] It is a double-stranded DNA (dsDNA) virus. It is characterized by species specificity and a slow replication cycle, often taking as long as 24 hours to produce virus progeny in infected cells and several days to weeks to produce visible cytopathic effects in laboratory cell lines. There is an icosahedral capsid, a tegument layer, a dense core surrounded by an amorphous matrix, and a lipid bilayer envelope with glycoproteins. There is a large dsDNA genome with 230 kilobases, which is organized into unique long (UL) and unique short (US) gene regions with internal and terminal repeats to enable four isomeric forms of the virus.[8] Cytomegalovirus gene products are, by convention, designated by whether these are encoded by the UL or US segment, and are numbered from "left-to-right."[9] Table 1 provides a detailed description of virus components.

Cytomegalovirus genome shows high diversity which is attributed to alternative splicing phenomena[9,10] and contains

[1]Department of Neonatology, Kailash Hospital, Noida, Uttar Pradesh, India

[2]Global Newborn Society (https://www.globalnewbornsociety. org/), Clarksville, Maryland, United States of America

[3]Department of Pediatrics, Louisiana State University, Shreveport, Louisiana, United States of America

[4]Department of Pediatrics, University of Alabama at Birmingham, Birmingham, Alabama, United States of America

Corresponding Author: Srijan Singh, Department of Neonatology, Kailash Hospital, Noida, Uttar Pradesh, India, Phone: +91 7011033174, e-mail: srijanstar89@gmail.com

How to cite this article: Singh S, Maheshwari A, Boppana S. CMV-induced Hearing Loss. Newborn 2023;2(4):249–262.

Source of support: NIH grant DC004163

Conflict of interest: Dr Akhil Maheshwari is associated as 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 the Editor-in-Chief and his research group.

Table 1: Major structural components of CMV

Structure	*Available information*
Lipid envelope	The lipoprotein envelope is derived from the nuclear membrane of an infected host cell and covers the nucleocapsid.[11] Cytomegalovirus has a characteristic three-layer architecture—an outer lipid bilayer envelope, inner nucleocapsid, and a middle tegument compartment
Glycoproteins	There are eight different glycoproteins embedded in the lipid bilayer.[11] Envelope surrounds the tegument and contains glycoproteins—the gB complex, the gM/gN complex, the gH/gL/gO "trimeric" complex, and the "pentameric complex" (PC) or pentamer comprising of proteins gH/gL/UL128, 130, and 131. Neutralizing antibodies targeting these glycoproteins are thought to be important component of protective immunity, hence recombinant forms of these proteins are candidates for vaccine development[12]
Receptor-binding motifs	Receptor-binding motifs are involved in virion attachment to host cell surface receptors during the process of infection and endocytosis. Cytomegalovirus utilizes binding to platelet-derived growth factor receptor alpha (PDGFRα) by glycoproteins gHgLgO (Trimer) and transforming growth factor beta receptor 3 (TGFβR3) to enter in multiple cell types.[13] The envelope proteins of the virus facilitate receptor binding by interacting with host cell proteins that act as binding factors and receptors—heparin sulfate proteoglycans (HSPGs), integrins, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), THY-1 cell surface antigen (CD90) neuropilin-2 (Nrp2), CD147, and OR14I1[14]
Envelope protein	The CMV envelope consists of various glycoprotein complexes that enable wide viral tropism[15] and facilitate attachment to cell surfaces and viral entry into the cells
Membrane protein	The human cytomegalovirus (HCMV) *UL50* gene encodes a transmembrane protein, pUL50, which acts as a core component of the nuclear egress complex (NEC) for nucleocapsids, which which facilitates capsid transport from the nucleus to the cytoplasm[16]
MHC or HLA proteins	Conserved B- and T-cell epitopes of CMV structural proteins may play an important role in evoking immune responses against CMV. Human-activated NK receptors also bind human CMV-encoded HLA class I-like molecules[17]
Spike protein	Glycoprotein gB is an elongated trimer and is similar to a spike with each protomer comprising 5 domains[18]
Viral tegument	In the mature virus particle, nucleocapsid is surrounded by tegument, a protein-rich layer containing several proteins which serve as targets of the host T lymphocyte response to infection, and hence are relevant to vaccine development. There is a high capsid pressure due to tightly packed, electrostatically repulsive genomic material in CMV, similar to HSV-1.[19,20] The β-herpesvirus–specific tegument protein pp150 contributes to a netlike tegument density layer stabilizing the capsid to facilitate the formation of infectious virions.[21–23] The tegument domain consists of approximately 30 proteins which play essential roles in the initial stages of infection following virus entry and late stages during virion assembly[24]
Capsid	Cytomegalovirus capsid has four parts—major capsid protein (MCP), triplex dimer (Tri2), triplex monomer (Tri1), and the small capsid protein (SCP).[25] Cytomegalovirus capsids have an icosahedral structure with major capsid protein (MCP) being organized in to 150 hexons and 11 penton vertices[26]
Capsomeres	Cytomegalovirus capsid has 162 capsomeres which function as structural subunits.[3] The capsid surrounds and encloses the viral dsDNA genome (forming a nucleocapsid) and can be seen as an electron-dense structure in electron micrographs
Protein core	There are 39 core proteins, which are present in all strains and are highly conserved.[27] Among the core proteins, some are involved in cell entry and immunomodulation/immunoevasion; while the function of 17 of them (UL10, UL139 or US33A) has not been determined
Enzymes	Cytomegalovirus has an essential, maturational serine protease whose catalytic domain, assembling (28 kDa), is released by self-cleavage from a 74-kDa precursor (pPR, pUL80a)[28]
RNA elements	Cytomegalovirus encodes for 26 mature microRNAs (miRNAs) that regulate transcriptions of both virus and host cells and to favor viral infection and inhibit the host's immune response Cytomegalovirus virion assembly involves the incorporation of RNA into infectious particles, which can be translated in newly infected cells in the absence of CMV genome transcription. It is also believed that nonspecifically incorporated cellular RNAs are crucial for virus assembly Immediate early (IE) mRNA is transcribed within the first few hours after infection of the host cell and the encoded IE proteins, which include multiple isoforms due to extensive splicing, modulate both host and viral gene expression
Nucleosome	The human CMV genome does not carry histones when encapsidated but nucleosomes are formed after release into the host cell nucleus. Initial nucleosome formation is genetically encoded at the human CMV major immediate early (IE) locus, but as infection proceeds to the late phase, nucleosomes redistribute extensively[29]
DNA	Human cytomegalovirus has a double-stranded DNA genome of approximately 236 kbp containing >170 open reading frames (ORFs) encoding functional proteins.[30] The virus encodes approximately 200 genes, including nine gene families, a large number of glycoprotein genes, and homologues of the human HLA class I and G protein-coupled receptor genes[31]
Genome-associated polyprotein	CMV UL80 ORF encodes protease and assembly protein from its N- and C-terminal regions, respectively and a 30-kDa protease is derived by autoproteolytic processing of a polyprotein which is the translation product of the entire UL80 ORF[32]
DNA polymerase	During the early phase of CMV infection *in vitro*, the virus DNA polymerase is rapidly induced.[33] It has immunologic specificity and is the target of the three drugs, ganciclovir, foscarnet, and cidofovir[34]
RNA polymerase	CMV utilizes RNA polymerase II to transcribe viral genes and produce viral mRNAs. RNA polymerase I (Pol I)-mediated transcription is active in the nucleolus[35]

HLA, human leukocyte antigens

many genes that enable the virus to evade host immune responses. Naturally acquired immunity does not protect against reinfection, thereby posing challenges in developing an effective vaccine.

Epidemiology

Congenital cytomegalovirus is the leading nongenetic cause of sensorineural hearing loss (SNHL), accounting for 6–30% of pediatric hearing loss.[36–41] In 1964, Medearis et al. described the association between cCMV and SNHL; they noted hearing impairment in more than 40% (2/5) of the survivors with disseminated cCMV, which was described as cytomegalic inclusion disease (CID).[42]

Cytomegalovirus transmission requires a close contact with body fluids. Infected infants and toddlers are the most important source of infection for women of child-bearing age.[43–45] Another common route of CMV transmission is via breast milk from seropositive mothers. Approximately, 85–90% of infants with cCMV have no clinical abnormalities at birth (asymptomatic cCMV), but 10–15% of these children go on to develop SNHL. Among children with symptomatic cCMV, 40–60% develop sequelae including SNHL, cognitive, motor, and vision deficits. In the United States, CMV contributes to 15–25% of childhood hearing loss.[46] Among infants with cCMV born to mothers with primary CMV infection during pregnancy, hearing loss and other neurologic sequelae are much more common in children whose mothers acquire primary infection in the first trimester as compared with later in pregnancy.[47–49] The incidence of SNHL in children with asymptomatic cCMV ranges between 6 and 25%[50–52] and 22–65% in those with symptomatic disease.[50]

The most important risk factors for SNHL are first trimester primary maternal infections, disseminated infection at birth, and neonatal imaging abnormalities. Other risk factors include using ototoxic drugs, longer NICU stay, fetal distress, and the need for mechanical ventilation during the neonatal period. These risk factors have been associated with SNHL independent of cCMV and therefore, are not very specific. However, the predictors of hearing loss in children with asymptomatic infection and those born following non-primary maternal infection are not known. Most infants with asymptomatic cCMV may not be recognized in a timely fashion because (a) there are no clinical findings at birth; (b) there is no routine screening for cCMV; and (c) it is difficult to collect saliva or urine samples after 2–3 weeks following birth.

In contrast to other congenital infections such as rubella and toxoplasmosis, the prevalence of cCMV increases with higher seroprevalence rates in the population. The incidence of studies from highly seropositive populations such as Brazil, India, and South Africa have demonstrated high prevalence of cCMV. The average prevalence of cCMV infections in high-income countries with low seroprevalence in women of child-bearing age is 0.64–0.7%, compared with 1–6% in resource-limited settings with high seroprevalence.[53–55] Although symptomatic cCMV was believed to occur exclusively following primary maternal infection, it is now clear that the frequency of symptomatic cCMV is similar in infected children born following both primary and non-primary maternal infections.[56] In addition, the frequency of SNHL in children with cCMV is also similar following primary and non-primary maternal infections. However, children with cCMV following primary maternal infection more frequently develop bilateral and more severe degree of SNHL than those born to mothers with non-primary maternal infection.

Both symptomatic and asymptomatic infants with cCMV shed virus in urine and saliva for prolonged periods, up to 6 years of age. Infants with symptomatic infections shed higher amounts of CMV in urine.[57] Some studies have suggested that higher blood CMV viral load may be a predictor of hearing loss,[57] but others have not confirmed these findings.[46,58,59] Noyola et al.[60] reported that hearing loss and progressive hearing loss was associated with a shorter period of CMV shedding. However, Rosenthal et al.[46] found that longer duration of viral shedding was associated with delayed onset hearing loss.[61]

In a prospective study of 14,000 unselected live-born infants spanning 10 years, the incidence of cCMV was noted as 0.53%, with 5.4% symptomatic cases.[62] Hearing loss was seen in 22% of the cCMV-infected infants (21% in asymptomatic and 33% in the symptomatic group). Hearing loss may deteriorate in two-third of symptomatic patients and in about 25% of children with asymptomatic cCMV.[63,64] Although the incidence of SNHL among infected children born to mothers with primary infection during pregnancy and those born to mothers with non-primary infection was similar, it has been suggested that bilateral and severe/profound loss occurs more often following maternal primary infection.[65] As we do not know the predictors of SNHL including progressive and severe/profound loss, current recommendations are to monitor all infected children with regular audiologic evaluations during early childhood, up to 4–6 years of age.[62] In a systematic review of 37 studies, the prevalence of cCMV in developed countries was estimated to be 0.58%. SNHL was noted in 12.6%, averaging around 1 out of 3 symptomatic children and 1 out of 10 asymptomatic children. Based on current data, 5 out of every 10,000 children born each year will develop cCMV-related hearing loss.[43] The degree of hearing loss is severe to profound in most affected children and in addition, many have a delayed onset, and progression of the deficit. Bilateral loss is more common among symptomatic children.

The Risk of cCMV Varies Based on Geographical Regions and CMV Seroprevalence

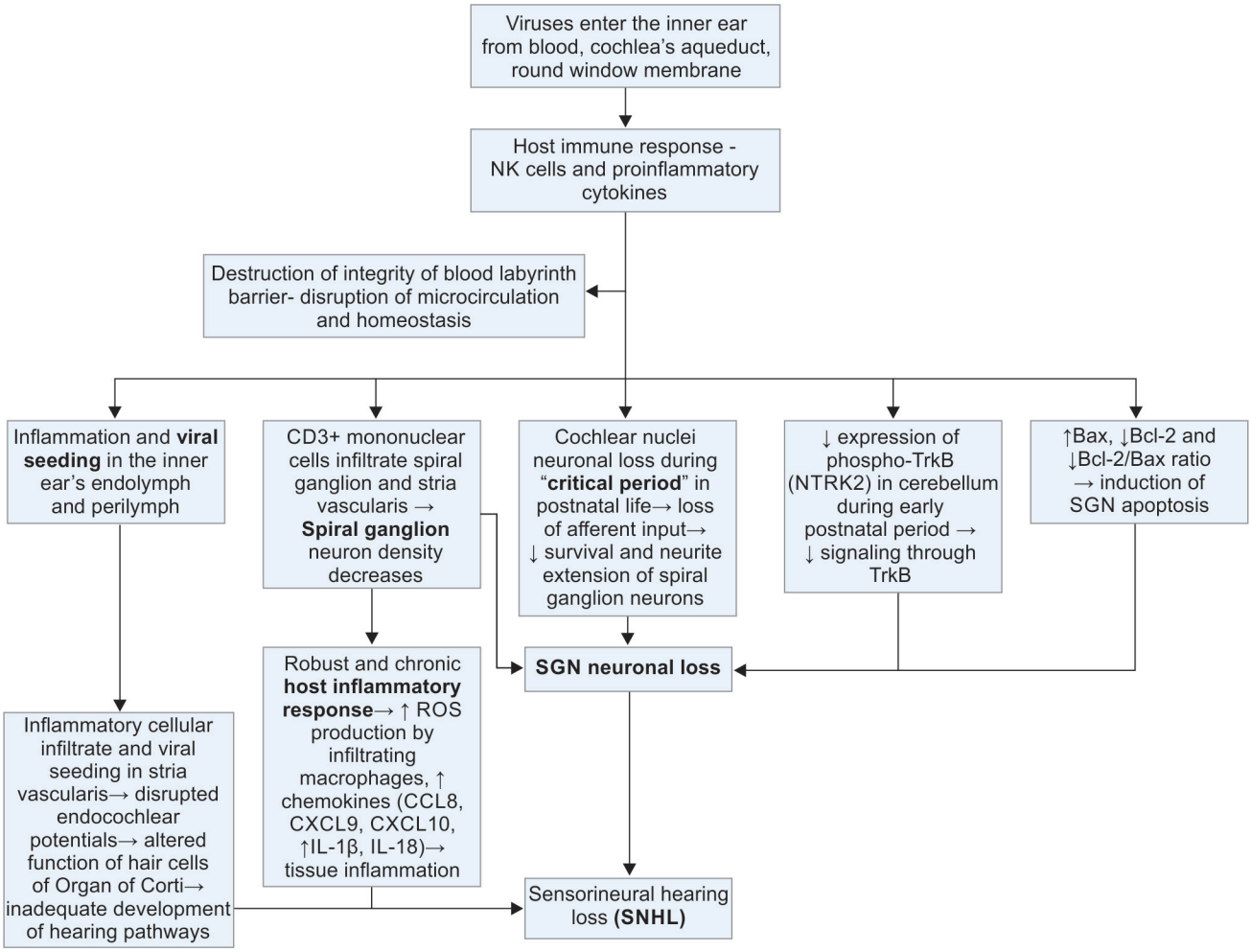
Worldwide, cCMV infection affects 0.2–2.5% of all live-born neonates.[61,66] Higher prevalence of cCMV infection is seen in populations with higher CMV seroprevalence rates.[57–59,67–69] In the United States, northern Europe, and other industrialized countries, 40–60% of the population shows CMV seroprevalence. The prevalence of cCMV is 0.64–0.7%. In contrast, near-universal seroprevalence rates have been observed in developing countries and the cCMV rates between 1 and 6% have been reported in these populations.[55,70] Population-based studies in Sweden,[71] Canada,[61] and the United States[50,72] have noted SNHL in 9.3–17% of infants with cCMV infections.[65]

Vertical Transmission

Cytomegalovirus-related hearing loss occurs following both primary (mother acquires the virus for the first-time during pregnancy) or non-primary maternal infection (seroimmune prior to pregnancy). In regions with high CMV, seroprevalence such as, Asia, South America, and Africa, most cCMV infections occur in children born to mothers with non-primary infections,[2] which is attributed to either reactivation of a latent virus or reinfection with new CMV strains. Although intrauterine transmission rate is higher in women with primary infections, vast majority of infected infants are born to mothers with non-primary infections.[43] Although the rate of vertical transmission is higher in women who acquire primary infection at later gestations, the risk of symptomatic infection and long-term sequelae are higher when maternal infection occurs during early gestation.

Birth prevalence of cCMV is directly proportional to maternal seroprevalence. High rates of non-primary infections also lead to a higher birth prevalence on a population level despite the lower risk of vertical transmission. Higher rates of CMV reinfections as

Flowchart 1: Pathogenesis of CMV-induced hearing loss



demonstrated by the acquisition of new serologic responses against strain-specific epitopes were observed in seropositive mothers with infected offspring.[73]

Most CMV-seropositive mothers (>90%) shed the virus in breast milk.[74] About 40–50% of exclusively breastfed infants of seropositive mothers acquire CMV infection during the first 4–6 months of life.[75,76] Although postnatal transmission of CMV via breastfeeding can lead to sepsis-like illness in very low birthweight infants, these children have not been noted to experience long-term sequelae that can be specifically attributed to CMV infections.

Pathogenesis

The pathogenesis of SNHL in children with cCMV is not well defined. Both virus-mediated direct cytopathic effects and inner ear inflammatory responses likely contribute to CMV-induced hearing loss.[77] In infants with symptomatic cCMV involving the central nervous system, treatment with 6 weeks of ganciclovir may reduce the risk of hearing deterioration at 6 months and possibly at 1 year of age.[67] However, one follow-up study comparing 6 weeks vs 6 months of valganciclovir in children with symptomatic cCMV showed no improvement in hearing in the short term; there was a modest improvement in hearing and developmental outcomes in the longer term.[68] During early stages of infection and viremia, CMV enters the inner ear from blood (the most important pathway of infection) or through cochlear aqueduct from subarachnoid space, and causes disruption of microcirculation, tissue hyperplasia in the organ of Corti, and cellular damage with loss of spiral ganglion neurons (SGNs) and changes in the endocochlear potential (EP) (Flowchart 1). The immune response induced by CMV infections including the activation of NK cells and increased expression of proinflammatory cytokines disrupt the blood–labyrinth barrier (BLB).[69,78,79] As cochlear implantation can improve hearing in most children with CMV-related SNHL, the neural pathways may be intact in most patients. However, the outcome following cochlear implantation in children with cCMV-related SNHL is more variable compared with children with SNHL due to other causes.[80–83]

A major barrier in understanding the mechanisms of cCMV-induced SNHL is the lack of small animal models. Recently, a murine model has been described where newborn mice infected with murine CMV (MCMV) develop disseminated viral infections including in the cochlea. These pups develop hearing loss similar to that seen in human infants with cCMV.[84,85] Findings in this model include hematogenous spread of the virus, induction of inflammatory responses, and the loss of spiral ganglion cells leading to increased auditory brainstem response (ABR) thresholds.[80,84,86,87] Reactive oxygen species (ROS)-induced inflammation contributes to hearing loss.[78,88] Activation of nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) in the cochlea and SGN

activates caspase 1 with consequent release of IL-1 beta and IL-18.[89] Chemokines, such as CCL8, CXCL9, and CXCL10 contributed to tissue inflammation in pups with ABR thresholds >60 dB. The cytopathology in the Organ of Corti was not prominent, but there was notable loss of SGN; increased ABR thresholds suggest that hearing loss may result from lesions in the auditory system other than hair cell loss.[78] Although viral antigens have been found in the inner ear,[76,90–92] the lack of significant inner ear histopathology along with persistence of inflammation in cochlea of mice with hearing loss indicates that inflammatory response and not direct virus-mediated cytopathology may play an important role in CMV-associated hearing loss.

Survival and neurite extension of the SGN is dependent on afferent input and on expression of the neurotrophins, brain-derived neurotrophic factor (BDNF) and NT3.[84,86,87,93] The loss of cochlear nuclei neurons may result in the loss of afferent input during critical period(s) prior to the onset of hearing.[80–82] Postnatal days 5–11 have been described as a critical period in mice; the ablation of the cochlea after postnatal day 14 does not result in neuronal loss in cochlear nuclei due to the acquisition of survival (antiapoptotic) functions in these cells.[83,85,94]

The potential mechanisms of CMV-related hearing loss may include (a) direct viral cytopathic effects; (b) immune response and inflammation leading to loss of SGN cells; (c) disruption of the BLB with damage in the *stria vascularis*, which is essential for maintaining EP; and (d) involvement of central auditory centers.

Direct Viral Cytopathic Effect

Early immune responses include activation of natural killer (NK) cells with increased expression of inflammatory cytokines and antibody-dependent cell-mediated cytotoxicity.[87,96]

Immune Response and Inflammation

The *stria vascularis* (SV) is critical for regulating the unique electrolyte composition of the extracellular fluid within the Organ of Corti and to maintain the EP,[97,98] which is the driving force for the transduction current in auditory hair cells.[99] It is maintained by high potassium levels in the endolymph.[100] Inflammatory cells and viral seeding can disrupt the SV[99,101] and consequently, the potassium cycle and EP.[101]

Disruption of Blood–Labyrinth Barrier Leading to Damage to the SV and Loss of EP

The cochlear BLB in the SV is paramount for the homeostasis of the cochlea.[102,103] Li et al. found higher BLB permeability following CMV infection due to disruption of the BLB, microcirculation, and the internal microenvironment.[69,85,104]

Involvement of Central Auditory Centers

SGNs are the first level of neurons of the auditory system; they receive electrical signal input from cochlear hair cells and transmit to the cochlear nucleus and thereafter to the auditory cortex[105] Cytomegalovirus may induce apoptosis in SGN cells[106,107] by via altered calcium homeostasis[81] or expression of Bax and Bcl-2.[106] Flowchart 1 summarizes the current understanding of the pathogenesis of CMV-induced hearing loss.

Viral reactivation and localized host inflammatory responses to reactivation might promote hearing loss as CMV, similar to other herpesviruses, establishes latency after primary infection.[60]

Clinical Presentation

About 10% of all infants with cCMV are symptomatic, and may present with hepatosplenomegaly, petechial, or purpuric rashes, jaundice with conjugated hyperbilirubinema, and/or microcephaly. The outcomes following cCMV infections are highly variable; most children with symptomatic cCMV develop sequelae such as SNHL, cerebral palsy, neurodevelopmental delay, and loss of vision.[70] About 50% of symptomatic neonates develop SNHL, of which two-thirds have neurologic deficits.[41,53,108,109]

About 10–15% of infants with asymptomatic cCMV with SNHL show permanent sequelae. Among infants with symptomatic infection, intrauterine growth retardation and petechiae are associated with the development of hearing loss. However, further study is needed to identify predictors of hearing loss in children with asymptomatic cCMV. CMV-associated SNHL can be delayed onset, progressive and fluctuating in children with both symptomatic and asymptomatic cCMV.[51,110–113] About half of the children with asymptomatic cCMV and hearing loss have bilateral impairment.[43,114]

Most infants with cCMV are not identified at birth because of the absence of clinical findings and because a significant proportion experience delayed onset and/or progressive SNHL, who are not identified with newborn hearing screening (NHS). Therefore, several strategies are being considered so that infected infants can be monitored closely for hearing loss and provide early intervention to improve outcomes. These strategies include screening of all newborns for cCMV (universal CMV screening) or CMV testing of all infants who fail their NHS (targeted CMV screening). In the United States, several states have enacted legislation mandating targeted CMV screening, CMV education during pregnancy or both. Currently, two of these states (Minnesota and New York) have implemented universal newborn CMV screening. As predictors of SNHL are not known, especially those with asymptomatic cCMV, all infected children should be monitored for hearing loss at least every 6 months through the 1st 5–6 years of age. Early detection and intervention during critical stages of speech and language function improves outcomes in children with CMV-associated hearing loss. Both primary and non-primary maternal CMV infections can lead to symptomatic cCMV infection and SNHL.[2] Although bilateral hearing loss is commonly associated with speech delay and is present in almost half of the cCMV-infected infants, recent studies have shown the adverse impact of unilateral SNHL on overall development.[111,112]

Delayed Onset, Progressive and Fluctuating Hearing Loss

Infants with cCMV can develop delayed onset and progressive SNHL during early childhood, which may continue to progress through adolescence.[41,61,115] The risk of developing SNHL after 5 years of age may not differ from that in uninfected children. Overall, 2% of the patients with SNHL require cochlear implantation.[116]

Children with cCMV have a higher probability of not passing their NHS (5–6%) compared with uninfected children (1–2%). However, a considerable proportion of children with CMV-associated SNHL will be missed on NHS because of delayed onset hearing loss and in some infants with mild hearing impairment.[117] Definitions of hearing loss, maternal infection, and neonatal infection are provided in Tables 2 to 4, respectively.

Diagnosis

Maternal Infection

Serological Testing

The presence of CMV IgG antibodies during pregnancy in previously seronegative individuals (seroconversion) is definitive evidence

Table 2: Definitions of hearing loss

Term	*Definition*
Conductive hearing loss	Hearing loss resulting from the disease process in the outer or middle ear that interferes with conduction of sound to the inner ear
Sensorineural hearing loss (SNHL)	Hearing loss due to damage, disease, or other disorders affecting the inner ear (eg., the cochlea) and/or the auditory nerve (cranial nerve VIII). Hearing loss is defined as sensorineural if the air-bone gap is <10 dB
Normal hearing	The ability to hear sounds between 0 and 20 dB
ABR threshold	The lowest intensity level at which wave V can be detected and replicated. An ABR click threshold >25 dB or a tone-pip threshold >30 dB is considered abnormal[118]
Mild hearing loss	Detection of sounds at 21–40 dB thresholds. A person with a mild hearing loss may hear some speech sounds but soft sounds are hard to hear[119]
Moderate hearing loss	Detection of sounds at 41–60 dB. A person with a moderate hearing loss may hear almost no speech when another person is talking at a normal level[119]
Severe hearing loss	Detection of sounds at 61–90 dB. A person with severe hearing loss will hear no speech of a person talking at a normal level and only some loud sounds[119]
Profound hearing loss	Detection of sounds only at 91 dB or greater. A person with a profound hearing loss will not hear any speech and is only able to hear very loud sounds[119]
Progressive SNHL	When children with SNHL either at birth or during early childhood experience a worsening of their hearing thresholds during later visits. (Deterioration in hearing of 10 dB or more at any 1 frequency on behavioral audiometry or ABR threshold, documented on two separate evaluations.[111,118] Fluctuating and progressive hearing losses are assigned only if there is no concurrent middle ear disease that might influence threshold variation
Fluctuating SNHL	A change to a worse or better hearing threshold between consecutive assessments: an absolute difference of ≥20 dB in ≥1 frequencies, ≥10 dB across any 2 or 3 adjacent frequencies, ≥10 dB in the average of the pure-tone thresholds at 0.5, 1, 2, and 4 kHz (4-frequency average), or a change from "hearing" to "no response" or vice versa at three adjacent frequencies.[116,120]
Improvement of hearing loss	Defined as a final threshold that is better by 10 dB or more compared with the initial threshold[26]
Stable SNHL	No change in hearing between two assessments
Late onset or delayed hearing loss	A child with normal hearing at birth, develops hearing loss at follow-up visits. Usually, there are one or more hearing evaluations with a normal threshold documented for each ear before detection of SNHL.
SNHL at isolated frequencies	Children with ≥25 dB hearing loss in any frequency without affecting the 4-frequency average
High-frequency hearing loss	A decrease in hearing at 4000, 8000, and 12,000 Hz frequencies only (or a combination of these).[111,118]

*dB-Decibel

Table 3: Definitions of maternal CMV infections[121]

Term	*Definitions*
Primary infection	When a maternal seroconversion in a CMV IgG-negative individual occurs during pregnancy or when the serological results are highly suggestive of a primary CMV infection (positive IgM and low IgG avidity antibodies)
Presumptive primary infection	Some of the maternal CMV infections may have occurred in the months immediately prior to conception and CMV IgM antibody may last up to 3–6 months. Hence, they are considered presumptive primary infections
Proven primary maternal CMV infection	Mothers who were CMV IgG antibody negative during the first trimester of pregnancy, and did not have subsequent serologic testing, yet gave birth to a congenitally infected newborn, are considered to have a proven primary maternal CMV infection. Primary maternal CMV infections. Both proven primary and presumptive primary maternal CMV infections are grouped together as primary maternal CMV infections
Recurrent, or non-primary maternal CMV infection	When a congenitally infected neonate is born to a mother showing seroimmunity for CMV in a serum sample obtained before conception or when the serum sample obtained in the first trimester has high IgG avidity antibody. It is also defined as the presence of CMV IgG antibody before pregnancy, or presence of CMV IgG and absence of CMV IgM antibody during first trimester. Non-primary infections during pregnancy could be due to reactivation of mother's endogenous strain or reinfection with a new strain of CMV
Uninfected maternal status	Defined as mothers who are CMV IgG seronegative in the first trimester and remain CMV IgG seronegative throughout pregnancy, and deliver an uninfected newborn
Unknown type of maternal CMV infection	Defined as the presence of CMV IgG antibody and the absence of CMV IgM antibody in the mother at delivery. Mothers whose serologic data are either incomplete or unavailable are also included in this category

of primary maternal CMV infection. However, early prenatal or preconceptional serum specimens are usually not available. Although the presence of CMV IgM antibodies indicates an acute infection, lower specificity of IgM assays and the presence of CMV IgM during reactivation or reinfection with a different virus makes the CMV IgM assays less reliable.[123] When both CMV IgG and

Table 4: Definitions of congenital/neonatal CMV infections[122]

Congenital CMV infection	Cytomegalovirus infection acquired *in utero*. Diagnosis can be made within the first three weeks of life by detection of CMV in newborn's urine or saliva
Postnatal CMV infection	Cytomegalovirus infection acquired in the postnatal period. After three weeks, CMV detection in urine or saliva may indicate either congenital or postnatal CMV infection. Postnatal CMV infection usually is clinically benign or self-limited
Symptomatic cCMV disease	Defined as a newborn with CMV detected in urine or saliva samples collected within 3 weeks of life, presenting with at least one of the clinical findings at birth: purpura/petechiae, jaundice, hepatosplenomegaly, microcephaly, unexplained neurological abnormality, elevated liver enzymes (alanine aminotransferase >100 IU), conjugated hyperbilirubinemia (direct bilirubin >2mg/dL), or thrombocytopenia (platelet count <100,000/mm^3)
Asymptomatic cCMV infection	Defined as a newborn with CMV detected in urine or saliva samples collected within 3 weeks of life, who has a normal newborn examination, that is, none of the symptoms defining symptomatic cases
Primary neurophenotype	Refers to patients with only central nervous system manifestations. They lack the typical somatic manifestations and may appear completely healthy at birth or may have microcephaly. On follow-up, they develop neurologic manifestations and neuroimaging shows polymicrogyria or other cortical dysplasia
Asymptomatic with isolated hearing loss	Refers to infants with isolated hearing loss at birth but no other symptoms. Categorization of these infants as "symptomatic" or "asymptomatic" is inconsistent, hence considered as a distinct category because they are not truly asymptomatic, but their disease is milder than that of symptomatic infants
Virologically confirmed congenital CMV infection	Diagnosed on the basis of any of the following: • Detection of CMV by viral culture in urine or saliva samples obtained within the first 3 weeks of life • Detection of CMV by shell vial assay in urine or saliva samples obtained within the first 3 weeks of life, with a positive confirmatory test (viral culture or PCR) • Detection of CMV via PCR in urine, saliva, or blood samples obtained within the first 3 weeks of life, confirmed on repeat testing • Detection of CMV via PCR in the newborn screening dried blood spot
Possible congenital CMV infection	A diagnosis of "possible" congenital CMV infection may be made if all of the following criteria are met: • One or more signs or symptoms of congenital CMV • Other conditions that cause these abnormalities have been excluded • Cytomegalovirus is detected in urine or saliva samples (via viral culture, shell vial assay, or PCR) or in the blood after the first three weeks of life
Not infected	Infants in whom CMV is not detected in urine or saliva (via viral culture, shell vial assay, or PCR) during the newborn period do not have congenital CMV. Because of the high sensitivity and specificity of these tests, a negative result excludes the diagnosis of congenital CMV infection. Congenital cytomegalovirus infection can be excluded beyond the newborn period if CMV IgG antibody testing is negative

IgM antibodies are present in a sample, IgG avidity testing could help differentiate between primary and non-primary maternal infection because affinity maturation of IgG antibodies usually takes several months after primary infection. The presence of IgM antibodies along with low-avidity IgG argues for a primary infection whereas high-avidity IgG suggests the likelihood of non-primary infections.[124,125]

Diagnosis of Fetal Infection

Ultrasound

Ultrasonographic features of fetal CMV infection include echogenic bowel, fetal edema, hepatomegaly, periventricular echo density, ventricular dilatation, cerebellar hypoplasia, and overall growth retardation.[126] However, these findings are seen in less than 25% of cCMV-infected fetuses and may also be found in other intrauterine infections and fetal diseases.[127]

Amniocentesis

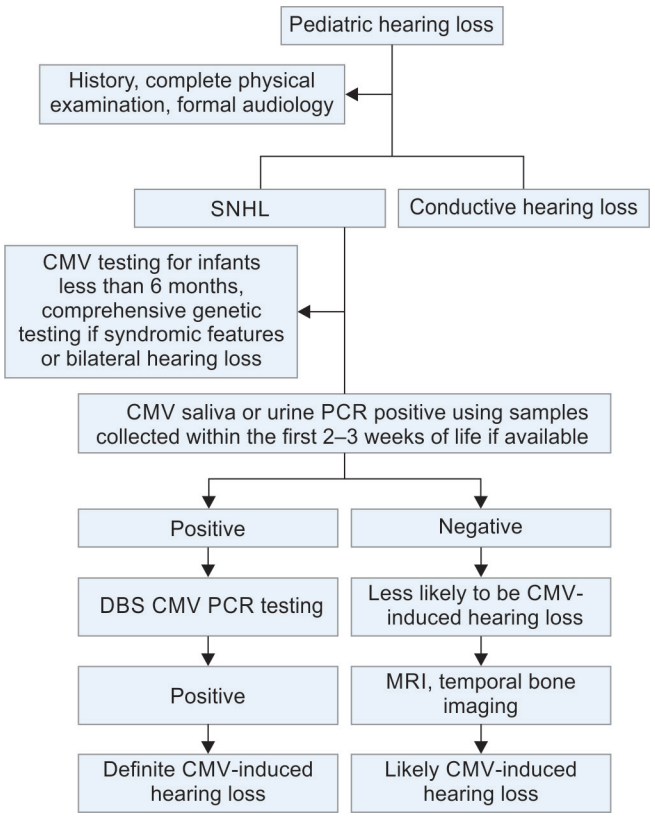
Amniotic fluid can be tested for CMV using virus culture and PCR to identify infected fetuses.[128] However, amniocentesis should be performed at least 6–7 weeks after primary maternal infection and after 20 weeks of gestation[108,129] because the appearance of viral particles in the amniotic fluid only occurs after the fetus begins to urinate. PCR using amniotic fluid is more sensitive (70–90%) than CMV cultures to diagnose fetal CMV infection.[130,131]

Diagnosis of cCMV in the Newborn Period

Most newborn infants with cCMV shed large amounts of virus in saliva and urine. The presence of infectious viruses, viral antigens, or viral DNA in saliva or urine samples confirms the diagnosis of cCMV (Flowchart 2). Since a substantial proportion of infants acquire CMV either from intrapartum exposure or postnatally from breastfeeding, it is important to test urine or saliva samples collected from infants within the first 2–3 weeks after birth to distinguish cCMV from a postnatal CMV infection. Postnatal infections can result in a sepsis-like syndrome in extremely premature infants and those with a primary immune deficiency such as severe combined immune deficiency. Postnatal CMV infection is not associated with long-term sequelae such as SNHL. Testing of newborn saliva samples using CMV PCR has been shown to be highly sensitive and specific.[132] To avoid contamination of saliva with CMV in breast milk from seropositive mothers, it is prudent to collect the saliva sample at least 90 minutes after breastfeeding. However, a large newborn screening study showed that false-positive saliva results are rare.[133]

As traditional culture methods are labor- and resource-intensive, and time-consuming, most clinical microbiology laboratories have phased out this test. In addition, culture-based assays are not suitable for screening large numbers of infants. In contrast, PCRs are less expensive with faster turn-around times, can be scaled up for high throughput capacity, and obviate the need to

Flowchart 2: Diagnostic algorithm of CMV-induced hearing loss



maintain tissue culture facilities. In addition, storage and transport conditions of samples usually does not affect the reliability of PCR results.[134–139]

Dried Blood Spot (DBS)

Testing of DBSs collected at the time of newborn metabolic screening for CMV allows retrospective diagnosis in children presenting with clinical findings or sequelae consistent with cCMV. However, there are some limitations such as lower sensitivity of PCR using DBS. Therefore, DBS CMV PCR cannot be used for mass screening for cCMV. The test does show high specificity (>99.9%) and can be useful in some instances.

Cranial Imaging

MRI brain can detect intracranial abnormalities in about a third of patients with probable or confirmed cCMV-induced SNHL. Brain ultrasound and/or MRI imaging findings in children with symptomatic cCMV include intracranial calcifications, migrational abnormalities, white matter disease, cerebral atrophy, ventriculomegaly, ventricular adhesions, and lenticulostriate vasculopathy.[140] However, many of these findings such as subependymal cysts and lenticulostriate vasculopathy are not as specific.

Other Evaluations

Ophthalmologic evaluation should be done to rule out chorioretinitis, optic atrophy, or retinal hemorrhages. However, eye findings are infrequent in children with asymptomatic cCMV.

Audiologic Evaluation

All newborns in the United States and most high-income countries undergo hearing screening prior to hospital discharge. More infants with cCMV fail NHS, about 5–6%, compared with 1–2% of uninfected children supporting the strategy that all babies who fail NHS should be tested for cCMV (hearing-targeted CMV screening). Although this approach identifies newborns with CMV-associated hearing loss but without clinical abnormalities, infected infants with asymptomatic cCMV who develop delayed onset hearing loss are not detected, arguing for universal newborn CMV screening. Cost-benefit analyses have shown that both hearing-targeted and universal CMV screening are cost-effective because identification of infants with cCMV and associated hearing loss will permit early intervention such as hearing amplification including cochlear implantation, antiviral therapy, and other measures to improve outcomes.[141]

In 2013, Utah became the first state to enact a CMV public health initiative on CMV education[11] and mandating CMV testing of all infants who fail NHS for CMV.[1,75,142] Many other states have enacted legislations mandating education and/or universal CMV screening; Minnesota and New York have recently implemented Universal newborn CMV screening.

CMV-associated SNHL has wide variability with respect to the severity of the loss, laterality, the time of onset, and the type of loss. There is no characteristic audiogram pattern seen in SNHL due to cCMV. Considering that nearly half of all children with cCMV and SNHL pass their NHS,[118] and with the lack of predictors or biomarkers to identify those at increased risk for delayed onset and/or progressive SNHL, there is a need to monitor hearing function in all infected children closely during first 4–5 years of age.[50,62]

Newborn hearing screening is carried out using either otoacoustic emission testing (OAE) or an automated auditory brainstem evoked response (ABR). In children who fail NHS, hearing loss should be confirmed by full-scale diagnostic ABR but unfortunately, ABR testing beyond neonatal age may require sedation. Visual reinforcement audiometry (VRA) can be used as early as 7 months of after birth. Audiologic evaluation in older child is performed in a soundproof environment using pure tone audiometry, speech audiometry, behavioral audiometry, visual reinforcement audiometry, play audiometry, impedance audiometry, tympanometry, and/or electrophysiologic tests (including auditory brainstem response and otoacoustic emissions).

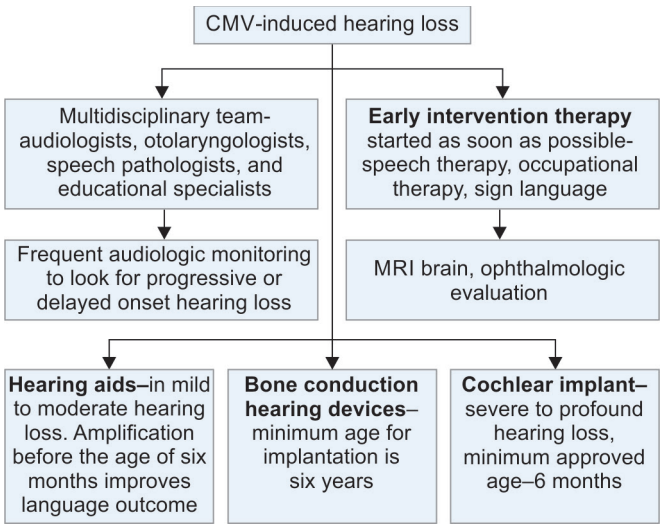
As with children with SNHL from other causes, children with CMV-associated hearing loss should also undergo genetic evaluation to identify the presence of an underlying genetic abnormality. Flowchart 2 describes the diagnostic algorithm for CMV-induced hearing loss.

Treatment

Antiviral Therapy

Ganciclovir and valganciclovir, nucleoside analogs, inhibit CMV replication by disrupting viral DNA synthesis.[143] A randomized controlled trial of intravenous ganciclovir for 6 weeks in infants with symptomatic cCMV with central nervous system involvement provided modest benefit by preventing progression of hearing loss and maintaining normal hearing. A subsequent study evaluated 6 weeks versus 6 months of oral valganciclovir therapy in children with symptomatic cCMV; although hearing and neurodevelopmental outcomes at 6 months were not different, the 6-month course showed significantly better outcomes at 1 and 2 years of age. Long-term follow-up studies still need to be performed and it is not known whether the benefits of antiviral therapy persist over time. The role of antiviral therapy in children with asymptomatic

Flowchart 3: Management of CMV-induced hearing loss



cCMV and those with mild symptomatic infection is not known and therefore, not recommended for these groups. Antiviral therapy is not recommended for preterm infants born those before 32 weeks of gestation because of the lack of pharmacokinetic data.[144]

Current guidelines for antiviral therapy in infants with moderate to severe symptomatic cCMV consists of a 6-month course of valganciclovir at 16 mg/kg/dose twice a day.[67] A complete blood count, transaminase levels, blood urea nitrogen (BUN) and creatinine should be done every 2–4 weeks during therapy. Children on treatment should be monitored for bone marrow suppression and in case of persistent neutropenia, valganciclovir should stopped temporarily. In addition, hepatic and renal function should be monitored.[73]

The management of cCMV-induced induced hearing loss has been summarized in Flowchart 3.

Multidisciplinary Approach

Children with hearing loss should be managed by a multidisciplinary team including audiologists, otolaryngologists, speech pathologists, clinical geneticists, genetic counsellors, and educational specialists. An ophthalmologic evaluation should be completed in all infected children. They should be referred to an early intervention services to meet the needs of hearing-impaired children including preferential seating or frequency-modulated (FM) systems at school. In children with early hearing loss, interventions including hearing amplification before the age of six months improves language outcome.[145]

Early Intervention Therapy

cCMV warrants periodic audiologic monitoring at 6-month intervals till 5 years of age, with frequent follow-ups 3 monthly when hearing levels are fluctuating. Frequent ear infections in young children lead to conductive hearing loss which superimposes SNHL leading to a delay in obtaining baseline audiologic data and requiring repeated follow-up assessments.

Hearing Aids

In-the-ear and in-the-canal hearing aids are appropriate only for hearing loss less than 60 decibels (dB). Digital and programmable hearing aids have better sound quality, increased precision, improved speech recognition.[146,147]

Assistive Listening Devices and Bone Conduction Hearing Devices

Bone-anchored implantable hearing aid system (BAHA) is feasible only in children 6 years of age or above because 3 to 4 mm of bone is needed to ensure osseointegration.[148]

Cochlear Implantation

Implantation at an early age ("critical period" of hearing development) provides better outcomes with bilateral implantation providing improved sound localization and ability to understand speech in noisy surroundings.[114,149] These management strategies have been approved by the US Food and Drug Administration for use in children as young as 12 months, although off-label use can be done in infants <12 months old.[150]

Hearing aids are recommended for children with unilateral or bilateral SNHL ≥40 db HL, and cochlear implants for those with bilateral SNHL ≥70 db HL. Around 5% of children with asymptomatic congenital CMV infection have SNHL ≥70 dB HL in at least 1 ear by age of 12 months, and half of these children meet current candidacy criteria for cochlear implantation.[116]

As we have not yet identified specific predictors of cCMV-induced SNHL, all infected children should be monitored with periodic audiologic evaluations to detect delayed onset and progressive hearing loss. Over 55% of the children will develop delayed onset loss occurring after the newborn period and 50% of all children with CMV-related SNHL will have progression or further deterioration of their loss overvtime.[50] In a prospective study conducted over 22 years, 5.7% of all cCMV-infected neonates ultimately required hearing amplification (hearing aid or cochlear implantation), with 44.4% of those with symptomatic infection and 3.4% of asymptomatic group requiring hearing rehabilitation.[140] Goderis et al. reported that there was a need for hearing amplification in 1.6% in children with asymptomatic and 29.3% in those with symptomatic infections.[151]

Unilateral hearing loss early in life can have deleterious effects on speech and language development and such children perform worse than their peers.[152,153] The term "aural preference syndrome" happens when a single-sided deafness in early childhood reorganizes the developing auditory pathways towards the hearing ear, with weaker central representation of the impaired side. Asymmetric hearing warrants a need for early, effective stimulation in both ears by appropriate fitting of auditory prostheses, including hearing aids and cochlear implants.[154]

Cytomegalovirus in blood is generally undetectable after one week of valganciclovir therapy. Continuous or intermittent detection of CMV at the age of 1 year has been seen in infants with SNHL. Cytomegalovirus load at diagnosis cannot predict the hearing outcome, but prolonged CMV viremia during treatment is a risk factor for SNHL and neurological sequelae.[155] Flowchart 3 demonstrates the management of CMV-induced hearing loss.

Prevention

In seronegative pregnant women, behavioral and hygiene precautions were effective in preventing primary maternal CMV infection.[43] The effectiveness of CMV hyperimmune globulin (HIG) to prevent intrauterine transmission of CMV in primary maternal infection has been investigated. Although non-randomized cohort studies have shown that HIG can prevent intrauterine transmission in mothers with primary infection, this benefit was not confirmed in the two randomized placebo controlled clinical

trials. Antiviral therapy with valacyclovir has shown promise in preventing intrauterine transmission in women with primary maternal infection.

CMV Hyperimmune Globulin

Cytomegalovirus-specific HIG therapy of pregnant patients with primary CMV infection in early pregnancy has been studied to prevent or reduce cCMV in offspring. In spite of the fact that earlier non-randomized studies have shown the efficacy of CMV HIG prophylaxis in primary maternal infection, the two randomized trials did not decrease the rate of cCMV in the HIG group compared with the placebo group.[156,157]

Vaccine Development

A report by the Institute of Medicine of the United States National Academy of Sciences designated that the development of a vaccine to prevent or reduce the adverse outcomes of cCMV is a priority.[158] Although a licensed CMV vaccine is not available, several candidate vaccines are currently in various stages of development.[159] In a phase 2 trial of an MF59-adjuvanted CMV glycoprotein B subunit vaccine in CMV seronegative women enrolled in the postpartum period, provided approximately 50% protection against acquiring primary infection.[160,161] However, the efficacy of the vaccine waned during the first 15 months of the study. The same vaccine given to seronegative teenagers failed to demonstrate protection from primary infection compared with placebo.[162] An mRNA-based vaccine expressing gB and the pentamer complex (mRNA-1647) examining the effectiveness of the candidate vaccine in preventing primary infection is currently in a phase 3 trial. A major challenge to the development of an effective vaccine is the fact that the majority of infants with cCMV are born to mothers with non-primary maternal infections. It is not known whether candidate vaccines that induce immune responses similar to those following natural infection will also provide protection against cCMV in infants born to seropositive women.

Prevention of Hearing Loss in cCMV-infected Children

Newborn hearing screening identifies about 50% of all infants with cCMV infection who have hearing loss.[163] A majority of children with CMV-associated SNHL experience progression of the deficit during early childhood. Among infants with cCMV who pass their NHS, about 5% will have delayed onset loss during early childhood. In addition, predictors or biomarkers of progressive and delayed onset SNHL, especially in children with asymptomatic cCMV have not been defined. Therefore, hearing function of all infected children should be monitored at least every 6 months during the first 4-5 years age and annually thereafter to detect progressive and/or delayed onset SNHL.

A National Institutes of Health consensus panel and the Joint Committee on Infant Hearing have endorsed a goal of universal detection of infants with hearing loss by 3 months of age.[164] Cytomegalovirus screening should be made an integral part of NHS program to achieve early detection and confirmation of hearing loss by 3 months of age and interventions for those with SNHL should begin by 6 months of age.[11]

Future Directions

Future efforts should be directed at elucidation of the mechanisms and pathogenesis of CMV-related hearing loss allowing for developing interventions to prevent or reduce this disability to develop support for newborn CMV screening programs, understanding the reasons for the failure of natural immunity to protect against reinfection/reactivation leading to cCMV, and the development of an effective vaccine to prevent or reduce the disease burden of cCMV including in highly seropositive populations and resource-limited settings.

Orcid

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

References

1. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. Rev Med Virol 2007;17(4):253–276. DOI: 10.1002/RMV.535.
2. Foulon I, Naessens A, Foulon W, et al. Hearing loss in children with congenital cytomegalovirus infection in relation to the maternal trimester in which the maternal primary infection occurred. Pediatrics 2008;122(6):e1123–e1127. DOI: 10.1542/PEDS.2008-0770.
3. Schleiss MR. Cytomegalovirus. Maternal Immunization 2020:253–288. DOI: 10.1016/B978-0-12-814582-1.00013-9.
4. Wilkinson GWG, Davison AJ, Tomasec P, et al. Human cytomegalovirus: Taking the strain. Med Microbiol Immunol 2015;204(3):273–284. DOI: 10.1007/S00430-015-0411-4/TABLES/1.
5. Martí-Carreras J, Maes P. Human cytomegalovirus genomics and transcriptomics through the lens of next-generation sequencing: Revision and future challenges. Virus Genes 2019;55(2):138–164. DOI: 10.1007/S11262-018-1627-3/TABLES/2.
6. Morton CC, Nance WE. Newborn hearing screening–A silent revolution. N Engl J Med 2006;354(20):2151–2164. DOI: 10.1056/NEJMRA050700.
7. Elek SD, Stern H. Development of a vaccine against mental retardation caused by cytomegalovirus infection in utero. Lancet 1974;1(7845):1–5. DOI: 10.1016/S0140-6736(74)92997-3.
8. Mocarski ES, Bonyhadi M, Salimi S, et al. Human cytomegalovirus in a SCID-hu mouse: Thymic epithelial cells are prominent targets of viral replication. Proc Natl Acad Sci USA 1993;90(1):104–108. DOI: 10.1073/PNAS.90.1.104.
9. Ma Y, Wang N, Li M, et al. Human CMV transcripts: An overview. Future Microbiol 2012;7(5):577–593. DOI: 10.2217/FMB.12.32.
10. Gatherer D, Seirafian S, Cunningham C, et al. High-resolution human cytomegalovirus transcriptome. Proc Natl Acad Sci USA 2011;108(49):19755–19760. DOI: 10.1073/PNAS.1115861108/SUPPL_FILE/SD01.XLS.
11. Schottstedt DrV, Blümel DrJ, Burger ProfDrR, et al. Human cytomegalovirus (HCMV) – Revised. Transfusion medicine and hemotherapy 2010;37(6):365. DOI: 10.1159/000322141.
12. Britt WJ, Mach M. Human cytomegalovirus glycoproteins. Intervirology 1996;39(5–6):401–412. DOI: 10.1159/000150510.
13. Kschonsak M, Rougé L, Arthur CP, et al. Structures of HCMV trimer reveal the basis for receptor recognition and cell entry. Cell 2021;184(5):1232–1244.e16. DOI: 10.1016/J.CELL.2021.01.036.
14. Stein KR, Gardner TJ, Hernandez RE, et al. CD46 facilitates entry and dissemination of human cytomegalovirus. Nature Commun 2019;10(1):1–13. DOI: 10.1038/s41467-019-10587-1.
15. Gardner TJ, Stein KR, Duty JA, et al. Functional screening for anti-CMV biologics identifies a broadly neutralizing epitope of an essential envelope protein. Nature Commun 2016;7(1):1–15. DOI: 10.1038/ncomms13627.
16. Lee MK, Hyeon S, Ahn JH. The human cytomegalovirus transmembrane protein pUL50 induces loss of VCP/p97 and is regulated by a small isoform of pUL50. J Virol 2020;94(13). DOI: 10.1128/JVI.00110-20.
17. Van Bergen J, Koning F. The tortoise and the hare: Slowly evolving T-cell responses take hastily evolving KIR. Immunology 2010;131(3):301. DOI: 10.1111/J.1365-2567.2010.03337.X.
18. Burke HG, Heldwein EE. Crystal Structure of the human cytomegalovirus glycoprotein B. PLoS Pathog 2015;11(10). DOI: 10.1371/JOURNAL.PPAT.1005227.

19. Yu X, Jih J, Jiang J, et al. Atomic structure of the human cytomegalovirus capsid with its securing tegument layer of pp150. Science 2017;356(6345). DOI: 10.1126/SCIENCE.AAM6892.
20. Bauer DW, Huffman JB, Homa FL, et al. Herpes virus genome, the pressure is on. J Am Chem Soc 2013;135(30):11216–11221. DOI: 10.1021/JA404008R.
21. Baxter MK, Gibson W. Cytomegalovirus basic phosphoprotein (pUL32) binds to capsids in vitro through its amino one-third. J Virol 2001;75(15):6865–6873. DOI: 10.1128/JVI.75.15.6865-6873.2001.
22. Yu X, Shah S, Lee M, et al. Biochemical and structural characterization of the capsid-bound tegument proteins of human cytomegalovirus. J Struct Biol 2011;174(3):451–460. DOI: 10.1016/J.JSB.2011.03.006.
23. Dai X, Yu X, Gong H, et al. The smallest capsid protein mediates binding of the essential tegument protein pp150 to stabilize DNA-containing capsids in human cytomegalovirus. PLoS Pathog 2013;9(8). DOI: 10.1371/JOURNAL.PPAT.1003525.
24. Terhune SS, Schröer J, Shenk T. RNAs are packaged into human cytomegalovirus virions in proportion to their intracellular concentration. J Virol 2004;78(19):10390. DOI: 10.1128/JVI.78.19.10390-10398.2004.
25. Li Z, Pang J, Dong L, et al. Structural basis for genome packaging, retention, and ejection in human cytomegalovirus. Nature Commun 2021;12(1):1–14. DOI: 10.1038/s41467-021-24820-3.
26. Borst EM, Harmening S, Sanders S, et al. A Unique role of the human cytomegalovirus small capsid protein in capsid assembly. mBio 2022;13(5). DOI: 10.1128/MBIO.01007-22/SUPPL_FILE/MBIO.01007-22-S0006.DOCX.
27. Mancebo FJ, Parras-Moltó M, García-Ríos E, et al. Deciphering the potential coding of human cytomegalovirus: New predicted transmembrane proteome. Int J Mol Sci 2022;23(5). DOI: 10.3390/IJMS23052768/S1.
28. Brignole EJ, Gibson W. Enzymatic Activities of Human Cytomegalovirus Maturational Protease Assemblin and Its Precursor (pPR, pUL80a): Maximal Activity of pPR Requires Self-Interaction through Its Scaffolding Domain. J Virol 2007;81(8):4091. DOI: 10.1128/JVI.02821-06.
29. Zalckvar E, Paulus C, Tillo D, et al. Nucleosome maps of the human cytomegalovirus genome reveal a temporal switch in chromatin organization linked to a major IE protein. Proc Natl Acad Sci USA 2013;110(32):13126–13131. DOI: 10.1073/PNAS.1305548110/-/DCSUPPLEMENTAL.
30. Hage E, Wilkie GS, Linnenweber-Held S, et al. Characterization of Human Cytomegalovirus Genome Diversity in Immunocompromised Hosts by Whole-Genome Sequencing Directly From Clinical Specimens. J Infect Dis 2017;215(11):1673–1683. DOI: 10.1093/INFDIS/JIX157.
31. Bankier AT, Beck S, Bohni R, et al. The DNA sequence of the human cytomegalovirus genome. DNA Seq 1991;2(1):1–11. DOI: 10.3109/10425179109008433.
32. Jones TR, Sun L, Bebernitz GA, et al. Proteolytic activity of human cytomegalovirus UL80 protease cleavage site mutants. J Virol 1994;68(6):3742. DOI: 10.1128/JVI.68.6.3742-3752.1994.
33. Wahren B, Eriksson B. Cytomegalovirus DNA polymerase inhibition and kinetics. Adv Enzyme Regul 1985;23(C):263–274. DOI: 10.1016/0065-2571(85)90051-2.
34. Fillet AM, Auray L, Alain S, et al. Natural polymorphism of cytomegalovirus dna polymerase lies in two nonconserved regions located between domains Delta-C and II and between Domains III and I. Antimicrob Agents Chemother 2004;48(5):1865. DOI: 10.1128/AAC.48.5.1865-1868.2004.
35. Kostopoulou ON, Wilhelmi V, Raiss S, et al. Human cytomegalovirus and herpes simplex type I virus can engage RNA polymerase I for transcription of immediate early genes. Oncotarget 2017;8(57):96536. DOI: 10.18632/ONCOTARGET.22106.
36. Diener ML, Zick CD, McVicar SB, et al. Outcomes From a Hearing-Targeted Cytomegalovirus Screening Program. Pediatrics 2017;139(2). DOI: 10.1542/PEDS.2016-0789.
37. Stehel EK, Shoup AG, Owen KE, et al. Newborn hearing screening and detection of congenital cytomegalovirus infection. Pediatrics 2008;121(5):970–975. DOI: 10.1542/PEDS.2006-3441.
38. Grosse SD, Ross DS, Dollard SC. Congenital cytomegalovirus (CMV) infection as a cause of permanent bilateral hearing loss: A quantitative assessment. J Clin Virol 2008;41(2):57–62. DOI: 10.1016/J.JCV.2007.09.004.
39. Barbi M, Binda S, Caroppo S, et al. A wider role for congenital cytomegalovirus infection in sensorineural hearing loss. Pediatr Infect Dis J 2003;22(1):39–42. DOI: 10.1097/00006454-200301000-00012.
40. Park AH, Duval M, McVicar S, et al. A diagnostic paradigm including cytomegalovirus testing for idiopathic pediatric sensorineural hearing loss. Laryngoscope 2014;124(11):2624–2629. DOI: 10.1002/LARY.24752.
41. Dahle AJ, Fowler KB, Wright JD, et al. Longitudinal investigation of hearing disorders in children with congenital cytomegalovirus. J Am Acad Audiol 2000;11(5):283–290. PMID: 10821506.
42. Hanshaw JB. Cytomegaloviruses. Infectious Diseases in Obstetrics and Gynecology, Sixth Edition. Published online January 1, 2008:48–56. DOI: 10.1007/978-3-662-39771-8_1/COVER.
43. Goderis J, De Leenheer E, Smets K, et al. Hearing loss and congenital cmv infection: a systematic review. Pediatrics 2014;134(5):972–982. DOI: 10.1542/PEDS.2014-1173.
44. Gaytant MA, Steegers EAP, Semmekrot BA, et al. Congenital cytomegalovirus infection: Review of the epidemiology and outcome. Obstet Gynecol Surv 2002;57(4):245–256. DOI: 10.1097/00006254-200204000-00024.
45. Cannon MJ, Hyde TB, Schmid DS. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. Rev Med Virol 2011;21(4):240–255. DOI: 10.1002/RMV.695.
46. Rosenthal LS, Fowler KB, Boppana SB, et al. Cytomegalovirus shedding and delayed sensorineural hearing loss: Results from longitudinal follow-up of children with congenital infection. Pediatr Infect Dis J 2009;28(6):515. DOI: 10.1097/INF.0B013E318198C724.
47. Faure-Bardon V, Magny JF, Parodi M, et al. Sequelae of congenital cytomegalovirus following maternal primary infections are limited to those acquired in the first trimester of pregnancy. Clin Infect Dis 2019;69(9):1526–1532. DOI: 10.1093/CID/CIY1128.
48. Stagno S, Pass RF, Cloud G, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. JAMA 1986;256(14):1904–1908.
49. Liesnard C, Donner C, Brancart F, et al. Prenatal diagnosis of congenital cytomegalovirus infection: Prospective study of 237 pregnancies at risk. Obstetrics and Gynecology 2000;95(6):881–888. DOI: 10.1016/S0029-7844(99)00657-2.
50. Fowler KB, Boppana SB. Congenital cytomegalovirus (CMV) infection and hearing deficit. J Clin Virol 2006;35(2):226–231. DOI: 10.1016/J.JCV.2005.09.016.
51. Iwasaki S, Yamashita M, Maeda M, et al. Audiological outcome of infants with congenital cytomegalovirus infection in a prospective study. Audiol Neurootol 2007;12(1):31–36. DOI: 10.1159/000096156.
52. Koyano S, Morioka I, Oka A, et al. Congenital cytomegalovirus in Japan: More than 2 year follow up of infected newborns. Pediatrics International 2018;60(1):57–62. DOI: 10.1111/PED.13433.
53. Dollard SC, Grosse SD, Ross DS. New estimates of the prevalence of neurological and sensory sequelae and mortality associated with congenital cytomegalovirus infection. Rev Med Virol 2007;17(5):355–363. DOI: 10.1002/RMV.544.
54. Lanzieri TM, Dollard SC, Bialek SR, et al. Systematic review of the birth prevalence of congenital cytomegalovirus infection in developing countries. Int J Infect Dis 2014;22:44. DOI: 10.1016/J.IJID.2013.12.010.
55. Ludwig A, Hengel H. Epidemiological impact and disease burden of congenital cytomegalovirus infection in Europe. Euro Surveill 2009;14(9):26–32. PMID: 19317969.
56. Townsend CL, Forsgren M, Ahlfors K, et al. Long-term outcomes of congenital cytomegalovirus infection in Sweden and the United Kingdom. Clin Infect Dis 2013;56(9):1232–1239. DOI: 10.1093/CID/CIT018.
57. Stagno S, Pass RF, Reynolds DW, et al. Comparative study of diagnostic procedures for congenital cytomegalovirus infection. Pediatrics 1980;65(2):251–257. DOI: 10.1542/PEDS.65.2.251.

58. Ross SA, Ahmed A, Palmer AL, et al. Newborn dried blood spot polymerase chain reaction to identify infants with congenital cytomegalovirus-associated sensorineural hearing loss. J Pediatr 2017;184:57–61.e1. DOI: 10.1016/J.JPEDS.2017.01.047.
59. Ross SA, Novak Z, Fowler KB, et al. Cytomegalovirus blood viral load and hearing loss in young children with congenital infection. Pediatr Infect Dis J 2009;28(7):588–592. DOI: 10.1097/INF.0B013E3181979A27.
60. Noyola DE, Demmler GJ, Williamson WD, et al. Cytomegalovirus urinary excretion and long term outcome in children with congenital cytomegalovirus infection. Congenital CMV Longitudinal Study Group. Pediatr Infect Dis J 2000;19(6):505–510. DOI: 10.1097/00006454-200006000-00003.
61. Saigal S, Lunyk O, Larke RP, et al. The outcome in children with congenital cytomegalovirus infection. A longitudinal follow–up study. Am J Dis Child 1982;136(10):896–901. DOI: 10.1001/archpedi.1982.03970460026006.
62. Foulon I, Naessens A, Foulon W, et al. A 10-year prospective study of sensorineural hearing loss in children with congenital cytomegalovirus infection. J Pediatr 2008;153(1):84–88. DOI: 10.1016/J.JPEDS.2007.12.049.
63. Williamson WD, Desmond MM, LaFevers N, et al. Symptomatic congenital cytomegalovirus. Disorders of language, learning, and hearing. Am J Dis Child 1982;136(10):902–905. DOI: 10.1001/archpedi.1982.03970460032007.
64. Pass RF, Stagno S, Myers GJ, et al. Outcome of symptomatic congenital cytomegalovirus infection: Results of long-term longitudinal follow-up. Pediatrics 1980;66(5):758–762.
65. Yamamoto AY, Mussi-Pinhata MM, Isaac MDL, et al. Congenital cytomegalovirus infection as a cause of sensorineural hearing loss in a highly immune population. Pediat Infect Dis J 2011;30(12):1043–1046. DOI: 10.1097/INF.0B013E31822D9640.
66. Hagay ZJ, Biran G, Ornoy A, et al. Congenital cytomegalovirus infection: a long-standing problem still seeking a solution. Am J Obstet Gynecol 1996;174(1 Pt 1):241–245. DOI: 10.1016/S0002-9378(96)70401-5.
67. Kimberlin DW, Lin CY, Sánchez PJ, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: A randomized, controlled trial. J Pediatr 2003;143(1):16–25. DOI: 10.1016/S0022-3476(03)00192-6.
68. Kimberlin DW, Jester PM, Sánchez PJ, et al. Valganciclovir for symptomatic congenital cytomegalovirus disease. N Engl J Med 2015;372(10):933–943. DOI: 10.1056/NEJMOA1404599.
69. Li X, Shi X, Qiao Y, et al. Observation of permeability of blood–labyrinth barrier during cytomegalovirus-induced hearing loss. Int J Pediatr Otorhinolaryngol 2014;78(7):995–999. DOI: 10.1016/J.IJPORL.2014.03.013.
70. Dollard SC, Schleiss MR, Grosse SD. Public health and laboratory considerations regarding newborn screening for congenital cytomegalovirus. J Inherit Metab Dis 2010;33(Suppl 2). DOI: 10.1007/S10545-010-9125-3.
71. Ahlfors K, Ivarsson SA, Harris S, et al. Congenital cytomegalovirus infection and disease in Sweden and the relative importance of primary and secondary maternal infections. Preliminary findings from a prospective study. Scand J Infect Dis 1984;16(2):129–137. DOI: 10.3109/00365548409087131.
72. Singh G, Gaidhane A. A review of sensorineural hearing loss in congenital cytomegalovirus infection. Cureus 2022;14(10). DOI: 10.7759/CUREUS.30703.
73. Duval M, Park AH. Congenital cytomegalovirus: What the otolaryngologist should know. Curr Opin Otolaryngol Head Neck Surg 2014;22(6):495–500. DOI: 10.1097/MOO.0000000000000104.
74. Hamprecht K, Maschmann J, Vochem M, et al. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet 2001;357(9255):513–518. DOI: 10.1016/S0140-6736(00)04043-5.
75. Stagno S, Reynolds DW, Pass RF, et al. Breast milk and the risk of cytomegalovirus infection. N Engl J Med 1980;302(19):1073–1076. DOI: 10.1056/NEJM198005083021908.
76. Brandt CT, Cayé-Thomasen P, Lund SP, et al. Hearing loss and cochlear damage in experimental pneumococcal meningitis, with special reference to the role of neutrophil granulocytes. Neurobiol Dis 2006;23(2):300–311. DOI: 10.1016/J.NBD.2006.03.006.
77. Schachtele SJ, Mutnal MB, Schleiss MR, et al. Cytomegalovirus induced sensorineural hearing loss with persistent cochlear inflammation in neonatal mice. J Neurovirol 2011;17(3):201. DOI: 10.1007/S13365-011-0024-7.
78. Bradford RD, Yoo YG, Golemac M, et al. Murine CMV-induced hearing loss is associated with inner ear inflammation and loss of spiral ganglia neurons. PLoS Pathog 2015;11(4):e1004774. DOI: 10.1371/JOURNAL.PPAT.1004774.
79. Almishaal AA, Mathur PD, Hillas E, et al. Natural killer cells attenuate cytomegalovirus-induced hearing loss in mice. PLoS Pathog 2017;13(8):e1006599. DOI: 10.1371/JOURNAL.PPAT.1006599.
80. Harris JA, Rubel EW. Afferent regulation of neuron number in the cochlear nucleus: cellular and molecular analyses of a critical period. Hear Res 2006;216–217(1–2):127–137. DOI: 10.1016/J.HEARES.2006.03.016.
81. Rubel EW, Fritzsch B. Auditory system development: Primary auditory neurons and their targets. Annu Rev Neurosci 2002;25:51–101. DOI: 10.1146/ANNUREV.NEURO.25.112701.142849.
82. Tierney TS, Russell FA, Moore DR. Susceptibility of developing cochlear nucleus neurons to deafferentation-induced death abruptly ends just before the onset of hearing. J Comp Neurol 1997;378(2):295–306. DOI: 10.1002/(sici)1096-9861(19970210)378:2<295::aid-cne11>3.0.co;2-r.
83. Harris JA, Iguchi F, Seidl AH, et al. Afferent deprivation elicits a transcriptional response associated with neuronal survival after a critical period in the mouse cochlear nucleus. J Neurosci 2008;28(43):10990–11002. DOI: 10.1523/JNEUROSCI.2697-08.2008.
84. Leake PA, Hradek GT, Hetherington AM, et al. Brain-derived neurotrophic factor promotes cochlear spiral ganglion cell survival and function in deafened, developing cats. J Comp Neurol 2011;519(8):1526–1545. DOI: 10.1002/CNE.22582.
85. Firbas W, Gruber H, Wicke W. The blood vessels of the limbus spiralis. Arch Otorhinolaryngol 1981;232(2):131–137. DOI: 10.1007/BF00505032/METRICS.
86. Miller JM, Le Prell CG, Prieskorn DM, et al. Delayed neurotrophin treatment following deafness rescues spiral ganglion cells from death and promotes regrowth of auditory nerve peripheral processes: Effects of brain-derived neurotrophic factor and fibroblast growth factor. J Neurosci Res 2007;85(9):1959–1969. DOI: 10.1002/JNR.21320.
87. Zhai SQ, Guo W, Hu YY, et al. Protective effects of brain-derived neurotrophic factor on the noise-damaged cochlear spiral ganglion. J Laryngol Otol 2011;125(5):449–454. DOI: 10.1017/S0022215110002112.
88. Zhuang W, Wang C, Shi X, et al. MCMV triggers ROS/NLRP3-associated inflammasome activation in the inner ear of mice and cultured spiral ganglion neurons, contributing to sensorineural hearing loss. Int J Mol Med 2018;41(6):3448–3456. DOI: 10.3892/IJMM.2018.3539/HTML.
89. Shi X, Qiu S, Zhuang W, et al. NLRP3-inflammasomes are triggered by age-related hearing loss in the inner ear of mice. Am J Transl Res 2017;9(12):5611–5618.
90. Teissier N, Delezoide AL, Mas AE, et al. Inner ear lesions in congenital cytomegalovirus infection of human fetuses. Acta Neuropathol 2011;122(6):763–774. DOI: 10.1007/S00401-011-0895-Y.
91. Klein M, Koedel U, Pfister HW, et al. Morphological correlates of acute and permanent hearing loss during experimental pneumococcal meningitis. Brain Pathol 2003;13(2):123–132. DOI: 10.1111/J.1750-3639.2003.TB00012.X.
92. Nadol JB, Hsu W. Histopathologic correlation of spiral ganglion cell count and new bone formation in the cochlea following meningogenic labyrinthitis and deafness. Ann Otol Rhinol Laryngol 1991;100(9 Pt 1):712–716. DOI: 10.1177/000348949110000904.
93. Ramekers D, Versnel H, Grolman W, et al. Neurotrophins and their role in the cochlea. Hear Res 2012;288(1–2):19–33. DOI: 10.1016/J.HEARES.2012.03.002.
94. Mostafapour SP, Del Puerto NM, Rubel EW. bcl-2 Overexpression eliminates deprivation-induced cell death of brainstem auditory

neurons. J Neurosci 2002;22(11):4670–4674. DOI: 10.1523/JNEUROSCI.22-11-04670.2002.
95. Lombardi G, Garofoli F, Stronati M. Congenital cytomegalovirus infection: Treatment, sequelae and follow-up. J Matern Fetal Neonatal Med 2010;23 Suppl 3(SUPPL. 3):45–48. DOI: 10.3109/14767058.2010.506753.
96. Hammer Q, Rückert T, Romagnani C. Natural killer cell specificity for viral infections. Nature Immunology 2018;19(8):800–808. DOI: 10.1038/s41590-018-0163-6.
97. Nickel R, Forge A. Gap junctions and connexins in the inner ear: Their roles in homeostasis and deafness. Curr Opin Otolaryngol Head Neck Surg 2008;16(5):452–457. DOI: 10.1097/MOO.0B013E32830E20B0.
98. Ciuman RR. Stria vascularis and vestibular dark cells: Characterisation of main structures responsible for inner-ear homeostasis, and their pathophysiological relations. J Laryngol Otol 2009;123(2):151–162. DOI: 10.1017/S0022215108002624.
99. Cohen-Salmon M, Regnault B, Cayet N, et al. Connexin30 deficiency causes instrastrial fluid-blood barrier disruption within the cochlear stria vascularis. Proc Natl Acad Sci USA 2007;104(15):6229–6234. DOI: 10.1073/PNAS.0605108104.
100. Mittal R, Aranke M, Debs LH, et al. Indispensable role of ion channels and transporters in the auditory system. J Cell Physiol 2017;232(4):743–758. DOI: 10.1002/JCP.25631.
101. Teissier N, Bernard S, Quesnel S, et al. Audiovestibular consequences of congenital cytomegalovirus infection. Eur Ann Otorhinolaryngol Head Neck Dis 2016;133(6):413–418. DOI: 10.1016/J.ANORL.2016.03.004.
102. Wu J, Han W, Chen X, et al. Matrix metalloproteinase-2 and -9 contribute to functional integrity and noise-induced damage to the blood-labyrinth-barrier. Mol Med Rep 2017;16(2):1731–1738. DOI: 10.3892/MMR.2017.6784/HTML.
103. Juhn SK, Rybak LP. Labyrinthine Barriers and Cochlear Homeostasis. Acta Otolaryngol 2009;91(1–6):529–534. DOI: 10.3109/00016488109138538.
104. Kimura RS, Nye CL, Southard RE. Normal and pathologic features of the limbus spiralis and its functional significance. Am J Otolaryngol 1990;11(2):99–111. DOI: 10.1016/0196-0709(90)90006-H.
105. Bailey EM, Green SH. Postnatal expression of neurotrophic factors accessible to spiral ganglion neurons in the auditory system of adult hearing and deafened rats. J Neurosci 2014;34(39):13110–13126. DOI: 10.1523/JNEUROSCI.1014-14.2014.
106. Li X, Shi X, Wang C, et al. Cochlear spiral ganglion neuron apoptosis in neonatal mice with murine cytomegalovirus-induced sensorineural hearing loss. J Am Acad Audiol 2016;27(4):345–353. DOI: 10.3766/JAAA.15061/BIB.
107. Schmutzhard J, Glueckert R, Pritz C, et al. Sepsis otopathy: Experimental sepsis leads to significant hearing impairment due to apoptosis and glutamate excitotoxicity in murine cochlea. Dis Model Mech 2013;6(3):745–754. DOI: 10.1242/DMM.011205.
108. Manicklal S, Emery VC, Lazzarotto T, et al. The "silent" global burden of congenital cytomegalovirus. Clin Microbiol Rev 2013;26(1):86–102. DOI: 10.1128/CMR.00062-12.
109. Boppana SB, Ross SA, Fowler KB. Congenital cytomegalovirus infection: Clinical outcome. Clin Infect Dis 2013;57 Suppl 4(Suppl 4):S178–S181. DOI: 10.1093/CID/CIT629.
110. Hicks T, Fowler K, Richardson M, et al. Congenital cytomegalovirus infection and neonatal auditory screening. J Pediatr 1993;123(5):779–782. DOI: 10.1016/S0022-3476(05)80859-5.
111. Fowler KB, McCollister FP, Dahle AJ, et al. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. J Pediatr 1997;130(4):624–630. DOI: 10.1016/S0022-3476(97)70248-8.
112. Williamson WD, Demmler GJ, Percy AK, et al. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. Pediatrics 1992;90(6):862–866.
113. Yow MD, Williamson DW, Leeds LJ, et al. Epidemiologic characteristics of cytomegalovirus infection in mothers and their infants. Am J Obstet Gynecol 1988;158(5):1189–1195. DOI: 10.1016/0002-9378(88)90252-9.
114. Puhakka L, Lappalainen M, Lönnqvist T, et al. Hearing outcome in congenitally CMV infected children in Finland – results from follow-up after three years age. Int J Pediatr Otorhinolaryngol 2022;156. DOI: 10.1016/J.IJPORL.2022.111099.
115. Ahlfors K, Ivarsson SA, Harris S. Report on a long-term study of maternal and congenital cytomegalovirus infection in Sweden. Review of prospective studies available in the literature. Scand J Infect Dis 1999;31(5):443–457. DOI: 10.1080/00365549950163969.
116. Lanzieri TM, Chung W, Flores M, et al. Hearing loss in children with asymptomatic congenital cytomegalovirus infection. Pediatrics 2017;139(3). DOI: 10.1542/PEDS.2016-2610/53745.
117. Fowler KB. Congenital Cytomegalovirus Infection: Audiologic Outcome. Clin Infect Dis 2013;57(Suppl 4): S182–S184. DOI: 10.1093/CID/CIT609.
118. Fowler KB, Dable AJ, Boppana SB, et al. Newborn hearing screening: Will children with hearing loss caused by congenital cytomegalovirus infection be missed? J Pediatr 1999;135(1):60–64. DOI: 10.1016/S0022-3476(99)70328-8.
119. Understanding Hearing Loss. Parent's Guide to Hearing Loss. CDC. Accessed August 16, 2023. https://www.cdc.gov/ncbddd/hearingloss/parentsguide/understanding/understandinghearingloss.html.
120. Konrad-Martin D, James KE, Gordon JS, et al. Evaluation of audiometric threshold shift criteria for ototoxicity monitoring. J Am Acad Audiol 2010;21(5):301–304. DOI: 10.3766/JAAA.21.5.3.
121. Demmler-Harrison GJ, Miller JA. Group O behalf of the HCCLS. Maternal cytomegalovirus immune status and hearing loss outcomes in congenital cytomegalovirus-infected offspring. PLoS One 2020;15(10). DOI: 10.1371/JOURNAL.PONE.0240172.
122. Congenital cytomegalovirus infection: Clinical features and diagnosis - UpToDate. Accessed August 16, 2023.
123. Prince HE, Lapé-Nixon M. Role of cytomegalovirus (CMV) IgG avidity testing in diagnosing primary CMV infection during pregnancy. Clin Vaccine Immunol 2014;21(10):1377–1384. DOI: 10.1128/CVI.00487-14.
124. Davis NL, King CC, Kourtis AP. Cytomegalovirus infection in pregnancy. Birth Defects Res 2017;109(5):336–346. DOI: 10.1002/BDRA.23601.
125. Navti OB, Al-Belushi M, Konje JC. Cytomegalovirus infection in pregnancy – an update. Eur J Obstet Gynecol Reprod Biol 2021;258:216–222. DOI: 10.1016/J.EJOGRB.2020.12.006.
126. Jückstock J, Rothenburger M, Friese K, et al. Passive immunization against congenital cytomegalovirus infection: Current state of knowledge. Pharmacology 2015;95(5–6):209–217. DOI: 10.1159/000381626.
127. Lipitz S, Hoffmann C, Feldman B, et al. Value of prenatal ultrasound and magnetic resonance imaging in assessment of congenital primary cytomegalovirus infection. Ultrasound Obstet Gynecol 2010;36(6):709–717. DOI: 10.1002/UOG.7657.
128. Mestas E. Congenital cytomegalovirus. Adv Neonatal Care 2016;16(1):60–65. DOI: 10.1097/ANC.0000000000000242.
129. Chiopris G, Veronese P, Cusenza F, et al. Congenital cytomegalovirus infection: update on diagnosis and treatment. Microorganisms 2020;8(10):1–17. DOI: 10.3390/MICROORGANISMS8101516.
130. Revello MG, Lilleri D, Zavattoni M, et al. Prenatal diagnosis of congenital human cytomegalovirus infection in amniotic fluid by nucleic acid sequence-based amplification assay. J Clin Microbiol 2003;41(4):1772–1774. DOI: 10.1128/JCM.41.4.1772-1774.2003.
131. Bodéus M, Hubinont C, Bernard P, et al. Prenatal diagnosis of human cytomegalovirus by culture and polymerase chain reaction: 98 pregnancies leading to congenital infection. Prenat Diagn 1999;19(4):314–317. DOI: 10.1002/(sici)1097-0223(199904)19:4<314::aid-pd542>3.0.co;2-h.
132. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. N Engl J Med 2011;364(22):2111. DOI: 10.1056/NEJMOA1006561.
133. Ross SA, Michaels MG, Ahmed A, et al. Contribution of breastfeeding to false-positive saliva polymerase chain reaction for newborn congenital cytomegalovirus screening. J Infect Dis 2018;217(10):1612–1615. DOI: 10.1093/INFDIS/JIY057.

134. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. JAMA 2010;303(14):1375–1382. DOI: 10.1001/JAMA.2010.423.
135. Pinninti SG, Ross SA, Shimamura M, et al. Comparison of saliva PCR assay versus rapid culture for detection of congenital cytomegalovirus infection. Pediatr Infect Dis J 2015;34(5):536–537. DOI: 10.1097/INF.0000000000000609.
136. Kadambari S, Williams EJ, Luck S, et al. Evidence based management guidelines for the detection and treatment of congenital CMV. Early Hum Dev 2011;87(11):723–728. DOI: 10.1016/J.EARLHUMDEV.2011.08.021.
137. Swanson EC, Schleiss MR. Congenital cytomegalovirus infection: New prospects for prevention and therapy. Pediatr Clin North Am 2013;60(2):335–349. DOI: 10.1016/J.PCL.2012.12.008.
138. Sahiner F, Cekmez F, Cetinkaya M, et al. Congenital cytomegalovirus infections and glycoprotein B genotypes in live-born infants: A prevalence study in Turkey. Infect Dis (Lond) 2015;47(7):465–471. DOI: 10.3109/23744235.2015.1018316.
139. Fowler KB, Boppana SB. Congenital cytomegalovirus infection. Semin Perinatol 2018;42(3):149–154. DOI: 10.1053/J.SEMPERI.2018.02.002.
140. Foulon I, De Brucker Y, Buyl R, et al. Hearing loss with congenital cytomegalovirus infection. Pediatrics 2019;144(2). DOI: 10.1542/PEDS.2018-3095.
141. Cannon MJ, Griffiths PD, Aston V, et al. Universal newborn screening for congenital CMV infection: What is the evidence of potential benefit? Rev Med Virol 2014;24(5):291. DOI: 10.1002/RMV.1790.
142. Reynolds DW, Stagno S, Hosty TS, et al. Maternal cytomegalovirus excretion and perinatal infection. N Engl J Med 1973;289(1):1–5. DOI: 10.1056/NEJM197307052890101.
143. Lim Y, Lyall H. Congenital cytomegalovirus – who, when, what-with and why to treat? J Infect 2017;74 Suppl 1:S89–S94. DOI: 10.1016/S0163-4453(17)30197-4.
144. Nicloux M, Peterman L, Parodi M, et al. Outcome and management of newborns with congenital cytomegalovirus infection. Arch Pediatr 2020;27(3):160–165. DOI: 10.1016/J.ARCPED.2020.01.006.
145. Yoshinaga-Itano C, Sedey AL, Coulter DK, et al. Language of early- and later-identified children with hearing loss. Pediatrics 1998;102(5):1161–1171. DOI: 10.1542/PEDS.102.5.1161.
146. Kuk FK, Kollofski C, Brown S, et al. Use of a digital hearing aid with directional microphones in school-aged children. J Am Acad Audiol 1999;10(10):535–548.
147. Bamford J, McCracken W, Peers I, et al. Trial of a two-channel hearing aid (low-frequency compression-high-frequency linear amplification) with school age children. Ear Hear 1999;20(4):290–298. DOI: 10.1097/00003446-199908000-00002.
148. Federspil PA, Tretbar SH, Böhlen FH, et al. Measurement of skull bone thickness for bone-anchored hearing aids: An experimental study comparing both a novel ultrasound system (SonoPointer) and computed tomographic scanning to mechanical measurements. Otol Neurotol 2010;31(3):440–446. DOI: 10.1097/MAO.0B013E3181D2775F.
149. Thomas JP, Neumann K, Dazert S, et al. Cochlear implantation in children with congenital single-sided deafness. Otol Neurotol 2017;38(4):496–503. DOI: 10.1097/MAO.0000000000001343.
150. Colletti L, Mandalà M, Colletti V. Cochlear implants in children younger than 6 months. Otolaryngol Head Neck Surg 2012;147(1):139–146. DOI: 10.1177/0194599812441572.
151. Goderis J, Keymeulen A, Smets K, et al. Hearing in children with congenital cytomegalovirus infection: Results of a longitudinal study. J Pediatr 2016;172:110–115.e2. DOI: 10.1016/J.JPEDS.2016.01.024.
152. van Wieringen A, Boudewyns A, Sangen A, et al. Unilateral congenital hearing loss in children: Challenges and potentials. Hear Res 2019;372:29–41. DOI: 10.1016/J.HEARES.2018.01.010.
153. Fitzpatrick EM, Gaboury I, Durieux-Smith A, et al. Auditory and language outcomes in children with unilateral hearing loss. Hear Res 2019;372:42–51. DOI: 10.1016/J.HEARES.2018.03.015.
154. Gordon K, Henkin Y, Kral A. Asymmetric hearing during development: The aural preference syndrome and treatment options. Pediatrics 2015;136(1):141–153. DOI: 10.1542/PEDS.2014-3520.
155. Kawada J ichi, Torii Y, Kawano Y, et al. Viral load in children with congenital cytomegalovirus infection identified on newborn hearing screening. J Clin Virol 2015;65:41–45. DOI: 10.1016/J.JCV.2015.01.015.
156. Revello MG, Lazzarotto T, Guerra B, et al. A randomized trial of hyperimmune globulin to prevent congenital cytomegalovirus. N Engl J Med 2014;370(14):1316–1326. DOI: 10.1056/NEJMOA1310214.
157. Hughes BL, Clifton RG, Rouse DJ, et al. A Trial of hyperimmune globulin to prevent congenital cytomegalovirus infection. N Engl J Med 2021;385(5):436–444. DOI: 10.1056/NEJMOA1913569.
158. Stratton KR, Durch JS, Lawrence RS. Vaccines for the 21st Century: A Tool for Decisionmaking. DOI: 10.17226/5501.
159. Boppana SB, van Boven M, Britt WJ, et al. Vaccine value profile for cytomegalovirus. Vaccine 2023;41(Suppl 2):S53–S75. DOI: 10.1016/J.VACCINE.2023.06.020.
160. Pass RF, Zhang C, Evans A, et al. Vaccine prevention of maternal cytomegalovirus infection. N Engl J Med 2009;360(12):1191–1199. DOI: 10.1056/NEJMOA0804749.
161. Dekker CL, Arvin AM. One step closer to a CMV vaccine. N Engl J Med 2009;360(12):1250–1252. DOI: 10.1056/NEJME0900230.
162. Bernstein DI, Munoz FM, Callahan ST, et al. Safety and efficacy of a cytomegalovirus glycoprotein B (gB) vaccine in adolescent girls: A randomized clinical trial. Vaccine 2016;34(3):313–319. DOI: 10.1016/J.VACCINE.2015.11.056.
163. Fowler KB, McCollister FP, Sabo DL, et al. A targeted approach for congenital cytomegalovirus screening within newborn hearing screening. Pediatrics 2017;139(2). DOI: 10.1542/PEDS.2016-2128.
164. Awad R, Oropeza J, Uhler KM. Meeting the Joint Committee on Infant Hearing Standards in a Large Metropolitan Children's Hospital: Barriers and Next Steps. Am J Audiol 2019;28(2):251–259. DOI: 10.1044/2019_AJA-18-0001.

REVIEW ARTICLE

Organic Acidemias: Clinical Presentation in Neonates

Monika Kaushal[1], Gayatri Athalye-Jape[2], Mohammad Mozibur Rahman[3], Mario Motta[4]

Received on: 10 November 2023; *Accepted on*: 05 December 2023; *Published on*: 05 January 2024

Abstract

Organic acidemias (OAs) are heritable genomic abnormalities characterized by the absence or defects in critical enzyme(s), which result in the accumulation of abnormal and toxic organic acid metabolites. These metabolites can be detected in blood and/or urine in high levels. Organic acidemias can have severe clinical manifestations, where most patients become symptomatic within the neonatal period or early infancy. Mildly affected cases may present later during adolescence or adulthood following decompensation during illness, following surgery, or with prolonged fasting. Acute clinical presentations include liver failure, lethargy, altered sensorium (encephalopathy), and/or seizures in the acute phase; subacute/delayed manifestations may include failure to thrive, developmental delay, and/or cardiomyopathy. In neonates, differential diagnoses include sepsis, metabolic disturbances, and intracranial bleeding. A high index of suspicion is essential for early, timely diagnosis. This article seeks to provide consolidated information on OAs, including pathogenesis, clinical presentation, diagnosis, and contemporary management.

Keywords: 2-methyl glutaconate, 2-oxo-3-methylvaleric acid, 2-oxoisocaproic acid,3-hydroxy propionate, 3-hydroxy-2-ethyl glutarate, 3-hydroxy-3-methylglutaryl-CoA, 3-hydroxybutyrate, 3-hydroxyisovaleric acid, 3-keto-2-methylbutyrate, 3-keto-2-methylvalerate-methyl citrate, 3-methyl crotonyl glycine, 3-methylcrotonyl-CoA carboxylase, 3-methylcrotonylglycinuria, 3-methylglutaconic aciduria, 3-OH propionic acid, Alanine, Alloisoleucine, Amish, Ammonium scavenger, Anion gap, Barth syndrome, Basal ganglion hyperdensity, Biotinidase, Biotinidase deficiency, Branched-chain amino acids, Branched-chain organic acidemias, Canadian Dariusleut-Hutterite ethnicity, Canavan disease, Carbonic anhydrase-5a deficiency, Cardiomyopathy, Carglumic acid, CblB – disease, CblC-disease, CblD disease, CBIE disease, CblF disease, CblG disease, D2-hydroxybutyric aciduria, DNAJC19 gene, Expanded newborn screening, Extrapyramidal movement disorders, Fatty acid oxidation disorder, Gas chromatography-mass spectrometry, Glutaric acidemia type 1, Glutarylcarnitine, Glutaryl-CoA dehydrogenase, Glycine, High ammonia, HMG CoA lyase deficiency, Holocarboxylase synthetase, Holocarboxylase synthetase deficiency, Hydroxycobalamin, *In vitro* fertilization, Isovaleric acidemia, Isovaleryl carnitine, Isovaleryl-CoA dehydrogenase, Isovaleryl glycine, Isovaleryl-2-methylbutyrylcarnitine, Isovalerylglycine, Ketosis, L2-hydroxybutyric aciduria, Lactate, LMBD1-gene, Metabolic conditions, Methyl citrate, Methylmalonate, Methylmalonic acidemia, MMAA gene, MMACHC gene, MMADHC gene, MTR gene, MTRR gene, Multiple carboxylase deficiency, *N*-aspartoacylase deficiency, Neonate, Neuro-ophthalmologic syndrome, Neutropenia, OPA3 outer mitochondrial membrane lipid metabolism regulator, Pancreatitis, Pancytopenia, Prolonged QT – interval, Propionic acidemia, Propionyl carnitine, Propionyl glycine, Pyruvate, Pyruvate dehydrogenase deficiency, Smell, Succinate, Sweaty feet, Tafazzin acetyltransferase, Tandem mass spectrometry, Testicular dysgenesis, Tetralinoleoyl cardiolipin.

Newborn (2023): 10.5005/jp-journals-11002-0080

Key Points

- Organic acidemias (OAs) are genetic abnormalities characterized by absent/defective enzyme(s) that are needed for metabolism of organic acids.
- In newborn infants, OAs typically present with liver failure, lethargy, altered sensorium (encephalopathy), and/or seizures. Less severely afflicted patients may show impaired growth and developmental delay.
- A high index of suspicion is essential for early diagnosis. Differential diagnoses may include sepsis with multi-system organ failure, other metabolic disturbances, and intracranial bleeding.
- This review article focuses on pathogenesis, clinical presentation, diagnosis and contemporary management of OAs.

Introduction

Organic acidemias are a cluster of heritable genomic metabolic conditions in which the absence or structural/functional abnormalities of critical enzymes lead to defect(s) in protein metabolism and accumulation of toxic, acidic intermediates (Fig. 1).[1,2] These organic acid metabolites can be detected in plasma and urine.[1] Most patients become symptomatic in the neonatal period or early infancy, but some milder forms of OAs may not present until adolescence or adulthood.[3,4] These patients are frequently misdiagnosed as having sepsis with multi-system organ failure, and if not treated promptly, show high mortality.[5]

[1]Department of Neonatologist, Emirates Specialty Hospital, Dubai, United Arab Emirates

[2]Neonatal Directorate, King Edward Memorial Hospital for Women, Subiaco, Western Australia; School of Medicine, University of Western Australia, Perth, Australia

[3]Institute of Child and Mother Health (ICMH), Dhaka, Bangladesh

[4]Department of Neonatology, Neonatology and Neonatal Intensive Care, AUSL di Bologna, Bologna, Italy

Corresponding Author: Monika Kaushal, Department of Neonatologist, Emirates Specialty Hospital, Dubai, United Arab Emirates, Phone: +503761828, e-mail: monikakaushal022@gmail.com

How to cite this article: Kaushal M, Athalye-Jape G, Rahman MM, *et al*. Organic Acidemias: Clinical Presentation in Neonates. Newborn 2023;2(4):263–278.

Source of support: Nil

Conflict of interest: None

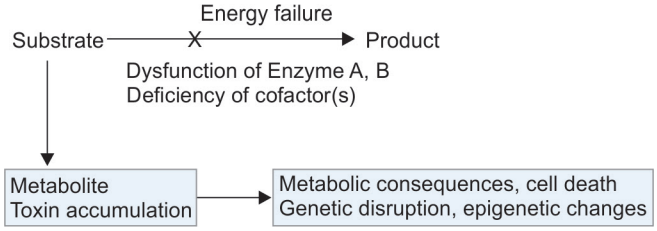


Fig. 1: Inborn errors of metabolism such as organic acidemias (OAs) become evident when dysfunctional metabolic pathways lead to accumulation of toxic metabolites

Classification

Organic aciduria are generally classified into three categories:[6,7]

1. Branched-chain organic acidemias:[8–13]
   a. Maple syrup urine disease (MSUD)
   b. Methylmalonic acidemia (MMA)
   c. Propionic acidemia (PA)
   d. Isovaleric acidemia (IVA)
   e. 3-methylcrotonylglycinuria (3-MCG)
   f. 3-methylglutaconic aciduria (3-MGA)
2. Multiple carboxylase deficiency[14–16]
   a. Holocarboxylase synthetase deficiency (HSD)
   b. Biotinidase deficiency
3. Cerebral organic acidemias:[17–21]
   a. Glutaric acidemia type 1 (GA1)
   b. *N*-Aspartoacylase deficiency (Canavan disease)
   c. 2-hydroxyglutaric acidurias

Clinical Features

There are two major clinical presentations of OAs.[22] The first, an intoxication type, does not interfere in the embryo-fetal period, and the neonate is normal at birth.[23] Most patients become symptomatic within the first 2 weeks after birth with poor feeding, vomiting, respiratory distress, rash, hypotonia, lethargy, and can progress to coma.[1,5] The most frequently seen acute presentations are feeding intolerance, liver failure, and altered sensorium.[24] Subacute/chronic manifestations include failure to thrive (FTT), developmental delay, and cardiomyopathy.[25] Metabolic crises may be precipitated during acute infections, following surgery, or with prolonged fasting.[26] Expanded newborn screening (NBS) has facilitated early, presymptomatic identification of affected infants and timely commencement of treatment.[27,28]

In older children and adults, OAs usually present with vomiting, lethargy, FTT, encephalopathy, and seizures.[29] Increased catabolism due to infections, surgery, or prolonged fasting may result in decompensation with severe metabolic acidosis and hyperammonemia.[5]

Diagnosis

Initial Evaluation

A high index of suspicion is essential for early diagnosis (Figs 2 and 3).[30] The presenting features may not be not so specific; these may include lethargy, poor feeding, vomiting, rash, failure to thrive, seizures, and hypotonia. A family history of consanguinity and neonatal deaths can be important.[31] A nonspecific skin rash, often erythematous and desquamating, can be seen in infants with HSD.[4] Patients with IVA and glutaric acidemia type II may have a "sweaty feet" odor.[32]

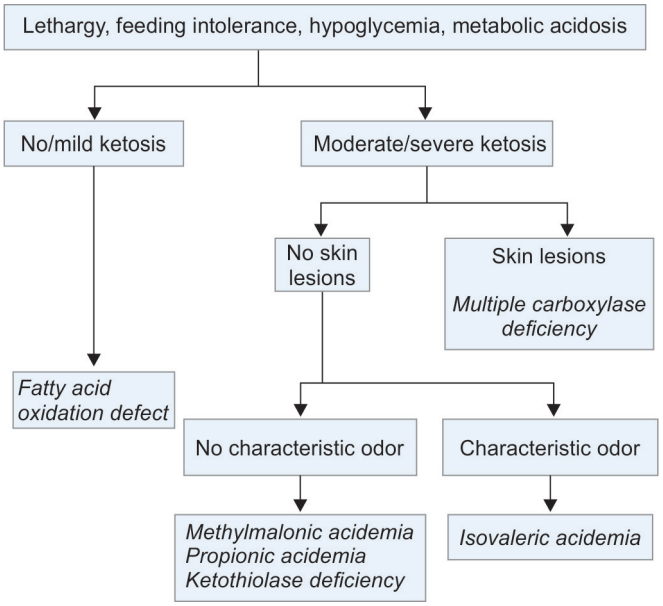


Fig. 2: Algorithm for diagnosing organic acidemias based on clinical characteristics

Differential diagnoses include Gram-negative sepsis, metabolic disturbances, and intracranial hemorrhage. Initial laboratory investigations should include evaluation for sepsis, with complete blood counts to look for neutropenia, thrombocytopenia, or pancytopenia. Metabolic parameters such as blood glucose, electrolytes, urea, creatinine, and blood gas panels for pH, carbon dioxide, bicarbonate, ammonia, lactate, and ketones.[4]

A test panel comprised of blood glucose levels, arterial blood gas, lactate, ammonia, and ketone levels, often labeled using the mnemonic GALAK, can be useful for identifying OAs.[33] Affected infants may show hypoglycemia or hyperglycemia, hypocalcemia, severe acidosis, increased anion gap, ketosis, high ammonia >200 µmol/L, mild–moderate rise in lactate levels, and neutropenia.[34,35] In older children, the ammonia levels may not be so high, rising only up to 90 µmol/L or so. In these patients, high lactate levels and neutropenia may be more useful as diagnostic indices. The investigations are usually performed in serial stages, starting from comprehensive screening to tests for specific disorders. A plasma amino acid, urine organic acid, enzyme assay, and genetic analysis is recommended.[36]

Gas chromatography-mass spectrometry (GC-MS) and tandem mass spectrometry (MS) can be useful for identifying/measuring organic acid levels in urine.[37] These tests may be further confirmed by molecular testing to identify compound heterogeneous or homogenous pathogenic mutations in respective disease genes.[38] Enzyme activities in lymphoblasts and fibroblasts can be used in some cases to confirm the diagnosis.[39]

MRI Brain

Bilateral basal ganglion hyperintensities, particularly in the caudate and/or lentiform nuclei, may indicate prior infarctions.[40] Cerebrospinal spaces and operculae may be wide in some patients.[41,42] White matter changes may also be seen.[17]

Antenatal Diagnosis

When a previous sibling has been diagnosed with an OA, parental counseling should be done regarding the possibility of recurrence

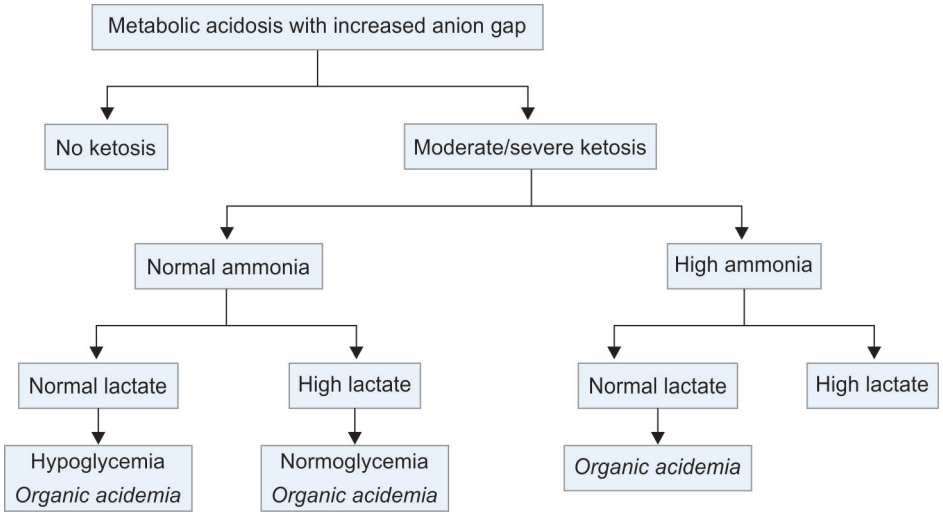


Fig. 3: Simplified laboratory approach for diagnosing organic acidemias

in the current fetus.[43] With those undergoing *in vitro* fertilization (IVF), preimplantation diagnosis could be made using molecular analysis if the parents' carrier status is known.[44] Another, although less accurate, way of prenatal diagnosis is to detect diagnostic compounds in amniotic fluid and enzyme activity in amniocytes or chorionic villi.[45,46] The delivery should be planned in a facility equipped to handle potential metabolic or other complications.[47]

After birth, expanded newborn screening should be sent at 36–48 hours to identify OAs.[48] If the tests show any suspicious results, plasma amino acid and urine OA levels can be useful for confirmation.

Differential Diagnosis

In neonates, sepsis due to gram-negative and other bacteria, viral infections, ductus-dependent congenital heart defects, neonatal abstinence syndromes, other inborn errors of metabolism, and congenital adrenal hyperplasia can mimic OAs.[1,4] In neonates with sepsis, abrupt onset and unexpectedly high levels of disease severity/systemic inflammatory response syndrome should raise concerns about the possibility of OAs. Severe acidosis with ketosis and less-than-anticipated lactate levels with hypoglycemia can help distinguish OA from other inborn errors of metabolism.[49] If there is hypoglycemia but no ketosis, diagnoses, such as fatty acid oxidation disorders, HMG CoA lyase deficiency, or pyruvate dehydrogenase deficiency should be considered.[50,51] If ketosis and lactate levels are too high, mitochondrial disorders without hyperammonemia should be considered.[52]

Management

Infants with OAs should be evaluated by a multidisciplinary team, including a biochemical geneticist, nephrologist, neurologist, and intensive care specialist.[5,53] The treatment should aim to (a) reduce production of the toxic intermediates by holding enteral intake for 24–48 hours and suppressing catabolism; (b) increase elimination of toxic metabolites; and (c) treat systemic complications.

During acute decompensation, the infant should be treated in an intensive care unit to evaluate for any metabolic decompensation. Metabolic acidosis, hyperammonemia, and any underlying infections should be treated. Infections should be treated aggressively. Catabolism can be reduced by providing sufficient calories; proteins should be reintroduced slowly after 48 hours.[5,34] A central venous line should be inserted for fluid management. Dextrose requirements may be high, electrolytes may need careful monitoring and supplementation. Intravenous lipids should be started immediately at levels around 2 gm/kg/day. There is a need to monitor acid–base balance; blood levels of electrolytes, glucose, and triglycerides; and complete blood counts with a particular focus on white cell count, and platelets. The glucose range should not exceed 130 mg/dL; some patients may show hyperglycemia and may need low-dose insulin infusions. Hydration and sodium bicarbonate may be needed to correct metabolic acidosis. Hyperammonemia may require treatment with an ammonium scavenger or carglumic acid.[54,55] In rare instances (ammonia >400 µmol/L), hemodialysis or hemofiltration can help. Peritoneal dialysis is not helpful.

Follow-up

After discharge from the intensive care unit, infants with OAs need continued follow-up by a pediatrician, biochemical geneticist, nephrologist, and a neurologist.[5,53]

Diet

A specific low-protein diet should be introduced gradually to promote growth and development, but these patients need close follow-up.[56] Natural protein intake is to be reduced; amino acid mixtures without offending amino acids should be used. High leucine levels can suppress growth. Carbohydrates and fat are needed for sufficient caloric administration.

Drugs

Metronidazole, and L-carnitine (100–300 mg/kg divided into three doses during decompensation) can be useful.[5]

Family Education

There is a need for clear, written instructions for routine care and management of emergencies. The mainstay of regular treatment at home is a high-carbohydrate diet, which should be administered even during the night. A diet-sheet is useful. An emergency-card with a list of warning signs and emergency diet should be provided.[57–60]

Table 1: Organic aciduria: enzyme deficiency, gene profile, plasma acylcarnitine profile, and organic acids excreted in urine

Organic acidemia	*Maple syrup urine disease*	*Methylmalonic aciduria*	*Propionic aciduria*	*Isovaleric aciduria*
Enzyme	Branched-chain alpha-keto acid dehydrogenase	Methylmalonyl-CoA mutase	Propionyl CoA carboxylase (PCC)	Isovaleryl–CoA dehydrogenase (IVD)
Genes	Branched-chain keto acid dehydrogenase E1, alpha polypeptide (BCKDHA), BCKDHB, or dihydrolipoamide branched-chain transacylase (DBT)	Methylmalonyl-CoA mutase	PCCA, PCCB	IVD
Plasma acylcarnitine profile	Elevated branched-chain amino acids Elevated alloisoleucine	Elevated propionylcarnitine (C3)	Elevated propionylcarnitine (C3)	Elevated isovaleryl carnitine
Urine organic acid analysis	2-Oxoisocaproic acid 2-Oxo-3-methylvaleric acid 2-Oxoisovaleric acid Succinate	2-Methyl glutaconate 3-Hydroxy propionate 3-Keto-2-methyl butyrate 3-Keto-2-methylvalerate, methylcitrate Methylmalonate 3-Hydroxybutyrate, propionyl glycine lactate, and pyruvate	2-Methyl glutaconate 3-Hydroxy-2-ethyl glutarate 3-Hydroxypropionate 3-Keto-2-methylbutyrate 3-Keto-2-methylvalerate-methyl citrate 3-Hydroxybutyrate, Propionyl glycine, lactate, and pyruvate	Hydroxyisovaleric acid Isovaleryl glycine

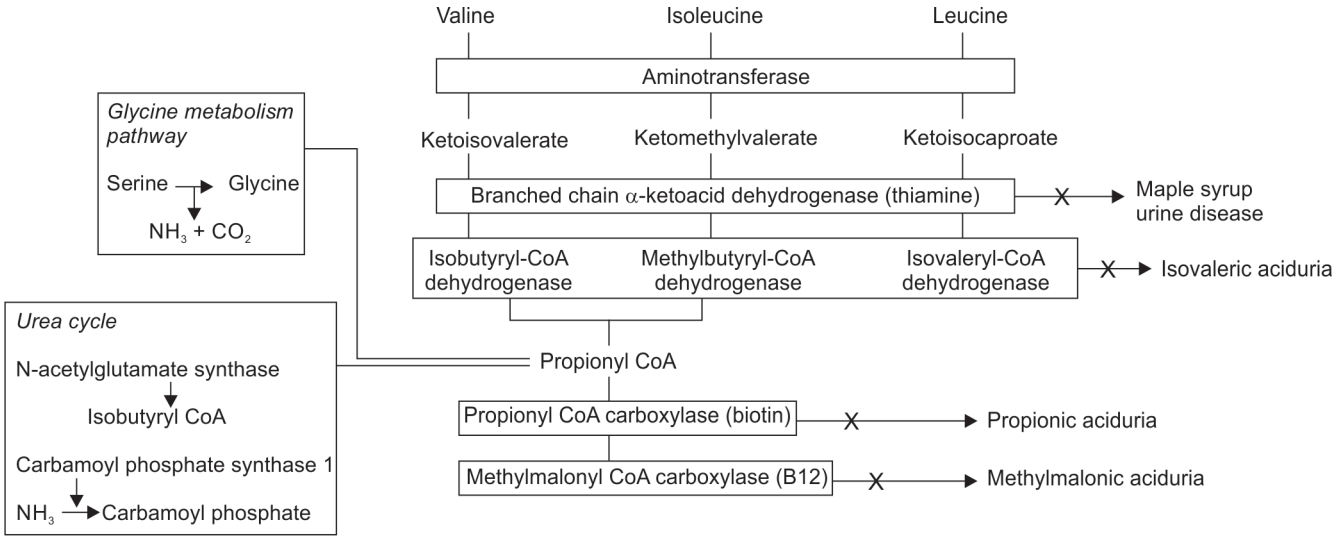


Fig. 4: Approach to identify branched-chain organic acidemias

Specific Disorders

1. Branched-chain Organic Acidemias (Details Provided in Table 1, Figs 3 and 4)

1A. Maple Syrup Urine Disease (MSUD)[61–65]

Maple syrup urine disease is an autosomal recessive (AR) disorder of branched-chain amino acid (BCAA) metabolism.[61] These enzymatic defects are localized to the branched-chain α-ketoacid dehydrogenase complex. Plasma levels of BCAAs and the pathognomonic disease marker, alloisoleucine, are elevated; so are urine concentrations of α-ketoacids.[66,67] The incidence is 1 out of 185,000 infants worldwide; the frequency is higher in certain ethnic groups such as the Mennonites in Pennsylvania and the Amish, in whom the disease could be seen in 1:200 infants.[63–65]

The condition is caused by AR mutations in genes branched-chain keto acid dehydrogenase E1 subunit alpha (BCKDHA), BCKDHB, dihydrolipoamide branched-chain transacylase E2 (DBT), and DLD genes.[68] The enzyme complex consists of four subunits, $E_1\alpha$, $E_1\beta$, E_2, and E_3, which activate the branch-chain aminotransferases.[61] These are essential for breaking down BCAAs, leucine, isoleucine, and valine, and the downstream ketoacid metabolites. The α-ketoacids, in turn, get converted into acetoacetate, acetyl-CoA, and succinyl-CoA through oxidative decarboxylation.

The name MSUD is derived from a distinctive sweet odor resembling maple syrup or fenugreek in the affected infants' urine and earwax, particularly during times of acute illness.[63] The compound responsible for the odor is 4,5-dimethyl-3-hydroxy-2[5H]-furanone (sotolon).[69]

The disorder is usually classified into the following subtypes:

- Classic MSUD, which manifests within the first 24–48 hours after birth.[62] The enzyme activity in these infants is <2% of

normal. Nearly, all patients show mutations in the E1 subunit of BCKD.[68] Clinical manifestations include poor feeding, irritability and/or lethargy, which might be followed by other neurologic signs, such as hypertonia, athetoid movements, opisthotonos, convulsions, and coma. If left untreated, these infants develop respiratory failure leading to death. In the medium term, these infants may lose bone mass, develop pancreatitis, intracranial hypertension, and developmental delay.[67]

- Intermittent MSUD: The enzyme activity in these patients is 5–20% of normal.[61,63,67] Patients show normal early development. Catabolic illness/stress can precipitate episodic ataxia/ketoacidosis with severe symptoms. The metabolic abnormalities may be missed on MS/MS NBS. The levels of BCAA and BCKA may be normal when asymptomatic.[70]
- Intermediate MSUD: The enzyme activity in these patients is 3–8% of normal.[67] The clinical features are first seen between the ages of 5 months and 7 years. These children usually do not show developmental delay but can become lethargic with maple syrup odor during physiological or infectious stress. In severe cases, metabolic crisis and neurological complications can occur.
- Late-onset MSUD: seen in adults; neurological signs included altered behavior, abnormalities of tone, ataxia, and seizures.[71] They may also develop hypoglycemia, ketoacidosis, and pancreatitis.
- Thiamine-responsive MSUD.[72] The enzyme activity in these patients can be 2–40% of normal. Clinically, these patients may resemble intermediate MSUD. Most patients have mutations in the E2 subunit of BCKDH.[68]
- Dihydrolipoamide dehydrogenase (DLD) deficiency.[73] Occasionally referred to as MSUD type 3 as DLD functions as the E3 subunit of BCKDH, a component of both pyruvate dehydrogenase and α-ketoglutarate dehydrogenase.[63] The enzyme activity in these patients can be 0–25% of normal. These infants may be normal on examination, or could show FTT, hypotonia, lactic acidosis, developmental delay, and/or abnormal movements.
- Those who cannot be easily classified into one of the above categories.

DNA testing is needed to ascertain the diagnosis.[63] It can be performed prior to birth.

Newborn screening: Newborn screening for MSUD using tandem mass spectrometry can show the blood concentrations of BCAAs such as leucine >1 mM/L within 2–3 days after birth.[74] The urine samples can be examined for branched-chain α-hydroxyacids and α-ketoacids.[61]

Treatment: There are four important steps:

1. Follow blood chemistries, including the levels of leucine, isoleucine, and valine.[61] Monitoring urine ketones can help when metabolic stress is suspected. Regular metabolic/full nutritional analysis can be helpful.
2. Use a tailored metabolic formula and adequate calories to prevent catabolism.[75] During periods of metabolic crisis, patients must be hospitalized for intravenous infusion of sugars and nasogastric drip-feeding of formula during periods of metabolic crisis. Diets with low levels of leucine, isoleucine, and valine can minimize neurological damage. Metabolic formula containing other essential amino acids, vitamins, minerals, ω-3 fatty acids, and trace elements are an essential aspect of MSUD management.[76] Specialized low-protein products, such as starch-based baking mixtures, imitation rice, and pasta may be prescribed, often alongside a protein-free carbohydrate powder added to food and/or drink. Patients with thiamine-responsive MSUD can have a higher protein intake diet with administration of high doses of thiamine (10–100 mg daily).
3. Removal of excess leucine reduces the impact of the disease on development.[67] Exchange transfusion, hemodialysis, or hemofiltration may be used.[77–79]
4. Liver transplantation can normalize metabolic function, particularly if it is performed at a young age.[80] Despite reducing the clinical manifestations, liver transplantation may not be curative; the patient will still carry two copies of the mutated BKAD gene in each of their own cells, which will consequently still be unable to produce the missing enzyme.

Prognosis: If left untreated, MSUD can be lethal due to central neurological disease and/or respiratory failure.[67] Most infants show some developmental delay.[81]

Management of mothers with MSUD: Metabolic control during pregnancy can be helpful for the fetus.[82] Dietary adjustments can reduce the incidence and severity of fetal growth/developmental abnormalities.

Future Studies:

- Gene therapy to overcome the genetic mutations that cause MSUD have been proven safe in animal studies. The administration of a viral vector (adeno-associated virus) containing an intact copy of the gene to express it in hepatocytes can restore normal metabolism.[83]
- Sodium phenylacetate/benzoate or sodium phenylbutyrate has been shown to reduce BCAA.[84] Phenylbutyrate can reduced serum BCAA and BCKA levels in many patients.

1B. Methylmalonic Aciduria (MMA)[5,85,86]

Methylmalonic aciduria is an AR condition with known mutations in the gene methylmalonyl-CoA mutase (MMUT; the suffix 0 suggests no residual function, Mut- indicates some residual activity).[9] There could be associated mutations in cobalamin metabolism; CblA disease involves the MMAA gene; CblB disease the MMAB; CblC-disease the MMACHC; CblD disease the MMADHC; CBlE disease the MTRR; CblF disease the LMBD1 gene; and the CblG disease is rooted in the MTR gene.[87] The LMBD protein is encoded by the gene LMBRD1 [limb region 1 (LMBR1) domain containing 1 gene (lmbrd1)];[88] it is involved in the conversion of vitamin B12 (also known as cobalamin) into one of two molecules, adenosylcobalamin (AdoCbl) or methylcobalamin (MeCbl).[89] adenosylcobalamin is required for the normal function of an enzyme known as methylmalonyl-CoA mutase.[90] The mitochondrial signaling pathways involved in MMA are summarized in (Fig. 5).

Clinical symptoms: Affected neonates may present with encephalopathy, especially following catabolic decompensation. Subacute illness presents with developmental delay, seizures, and extrapyramidal movement disorders. Optic atrophy /blindness, myelopathy (paresis), chronic kidney dysfunction, cardiomyopathy (cardiac dysfunction, arrhythmias with prolonged QT interval), and pancreatitis may be seen.[91]

Diagnosis: Acidosis and hyperammonemia indicate the possibility of MMA. Some patients show vitamin B12-responsiveness, where intramuscular or intravenous administration of 1 mg/dL hydroxycobalamin on 3 consecutive days leads to better levels of organic acids in urine (lowers to <50%), homocysteine in blood, and C3 carnitine in dried blood.[5,26,34,91] Neuroimaging can be helpful. Ventricular dilatation, cerebral atrophy, white matter changes, and corpus callosal thinning are frequently seen (Fig. 6).

Management: In emergency treatment of MMA, protein administration must be stopped immediately, and parenteral glucose and electrolytes should be started. Insulin infusion may be needed. Metabolic acidosis should be corrected using fluid infusions and sodium bicarbonate. Intravenous carnitine (250–500 mg/kg/day) along with vitamin B12 (1–2 mg/day) can be helpful. In infants with hyperammonemia, carglumic (50–100 mg/kg/day) should be started. If it is not effective, extracorporeal detoxification may be needed.[5,26,34,91]

After stabilization, a low-protein diet supplemented with amino acid mixture devoid of valine and isoleucine is recommended.[9] Catabolism is to be avoided.[26] Carnitine supplementation (50–100 mg/kg/day orally), and metronidazole (10–20 mg/kg/day) for 10 days per month can be useful. If metabolic acidosis does not get corrected with fluids, it is prudent to start sodium bicarbonate. In multivariate and disorders of cobalamin metabolism, vitamin B12 (hydroxycobalamin) injections intramuscular are given. In cases with anemia, erythropoietin may be considered although it might not be effective. Liver transplant is usually the only long-term solution.[92]

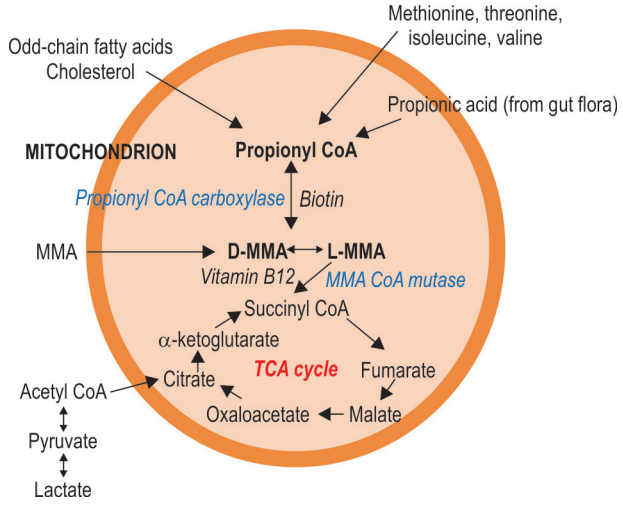


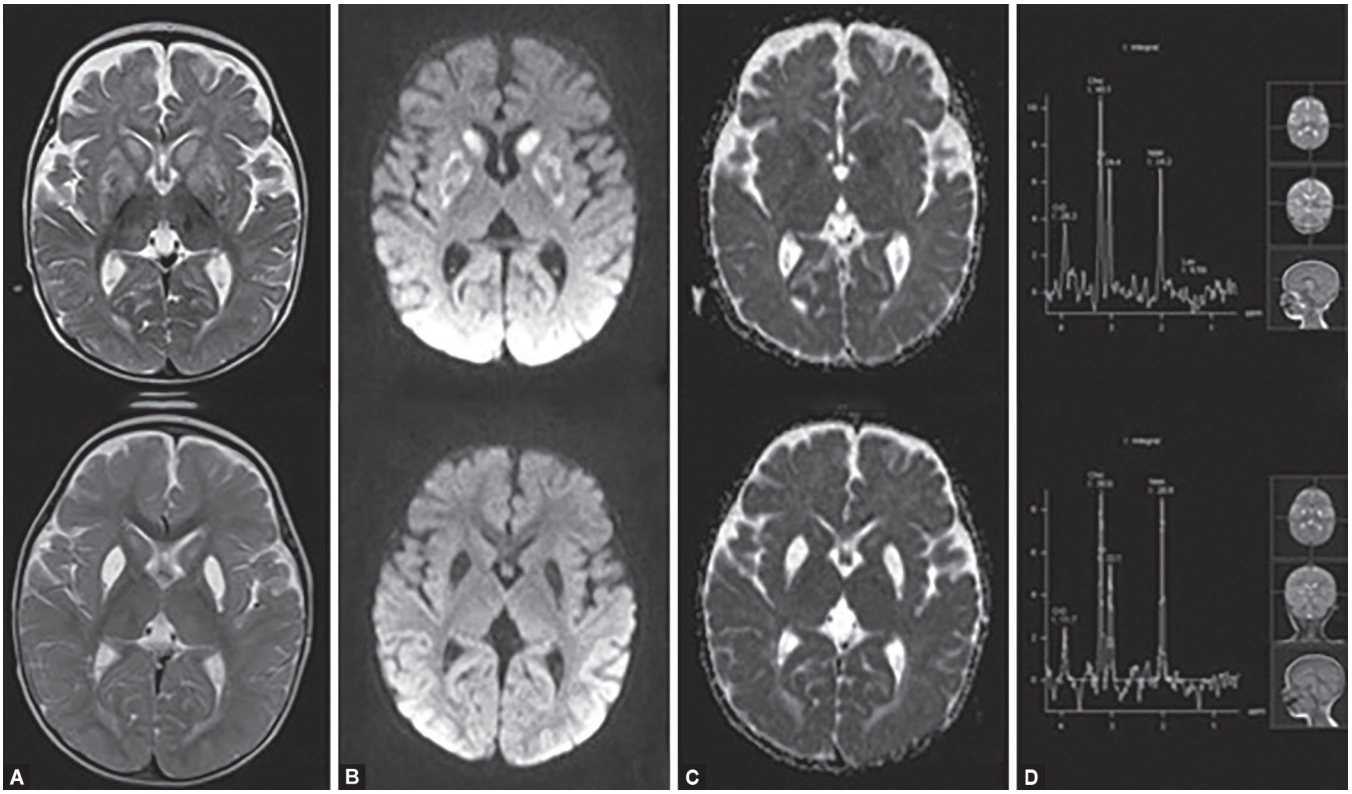
Fig. 5: Signaling pathways involved in the pathogenesis of methylmalonic acidemia (MMA)

Monitoring: Periodic measurements of organic acids in urine, homocysteine in blood (in CBI variants), amino acid profile in blood, and acylcarnitine profile in dried blood, including free carnitine, are indicated. Ammonia, lactate, blood counts, and kidney functions should be monitored closely.[93]

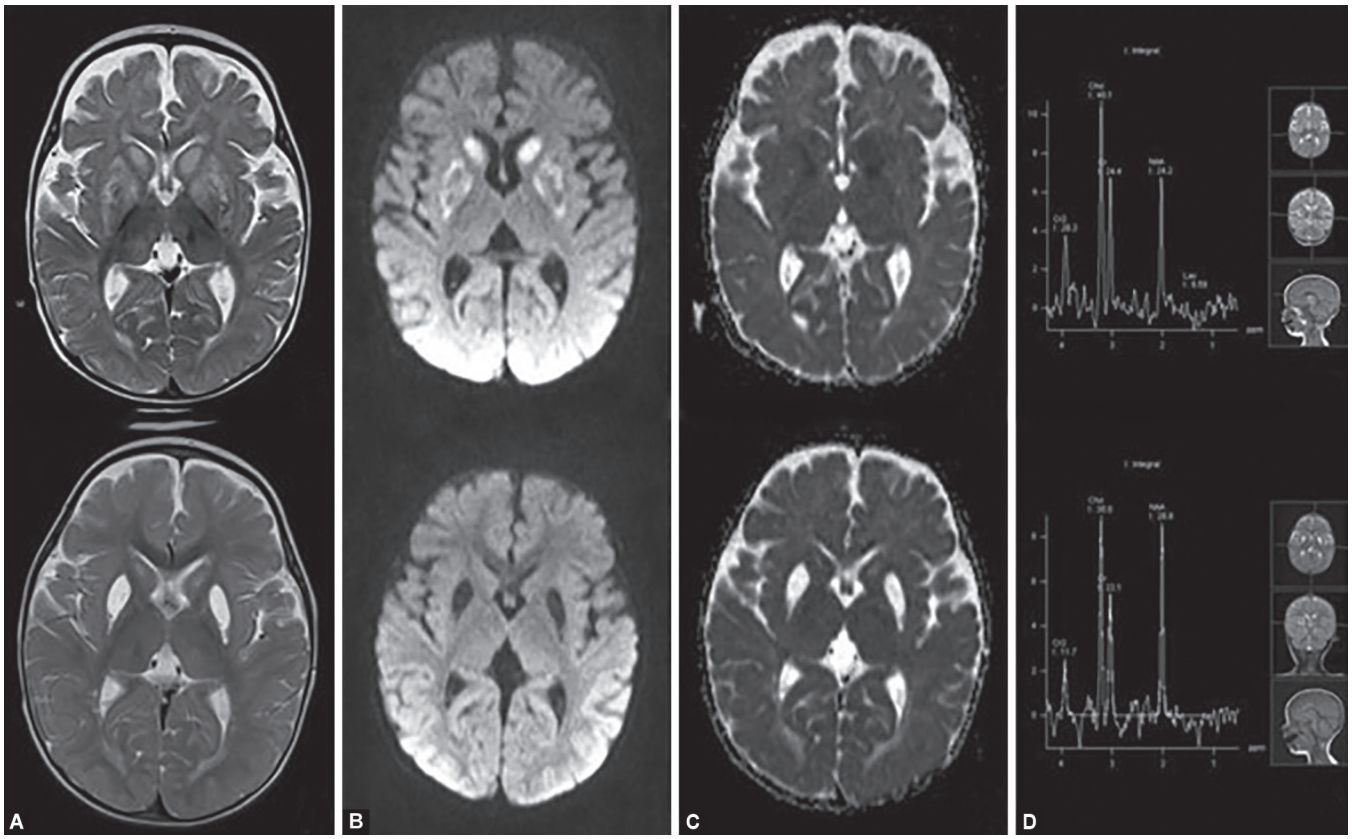
The outcome is guarded.[94] However, many cases with cobalamin-responsive subtypes may have better prognosis.[9,85]

1C. Propionic Acidemia (PA)[26,57]

Propionic acidemia (PA) is an AR condition with a mutation in the propionyl CoA carboxylase (PCC), including the PCCA or PCCB genes.[95–97] Each consists of two subunits with cofactor biotin.[10]



Figs 6A to D: MRI findings in an infant with methylmalonic acidemia (MMA). In MMA, ventricular dilatation, cerebral atrophy, white matter changes, and corpus callosal thinning are frequently seen



Figs 7A to D: MRI findings in an infant with propionic acidemia. Neuroimaging typically shows delayed myelination, cerebral atrophy, white matter lesions, and lesions in the globus pallidus

Clinical symptoms: Early-onset PA presents with encephalopathy especially following metabolic decompensation with developmental delay, cardiomyopathy, pancytopenia, anemia, thrombocytopenia, leukopenia hypotonia, lethargy, seizures, and coma.[10]

Late-onset PA presents mainly with neurological symptoms such as encephalopathy following metabolic decompensation, developmental delay, hypotonia, lethargy, seizures, and coma.[10,98] Some patients may show intermittent ataxia, altered behavior, and feeding difficulties. Pancytopenia, cardiomyopathy, and pancreatitis may be noted, although less frequently.

Diagnosis: The acronym GALAK, combining hypoglycemia, increased ammonia, lactic acidosis with an increased anion gap, acidosis, and ketonuria, provides a useful approach to diagnosis. The organic acids in urine will be elevated, especially 3-hydroxy propionic acid and methyl citrate, C5- and C6-ketoacids. Serum glycine and alanine may be elevated along with low free carnitine. Mutation analysis should be done. Neuroimaging can also provide helpful clues, with delayed myelination, cerebral atrophy, white matter lesions, and lesions in the globus pallidus (Fig. 7).

Differential diagnosis: Similar clinical manifestations may be seen in biotinidase deficiency and carbonic anhydrase-5α deficiency.[99,100]

Management: In ill-appearing infants with acute presentation, it is safer to stop oral diet and protein administration, and start parenteral infusion of glucose and electrolytes. Insulin may be added if required. Acidosis should be corrected with fluids and sodium bicarbonate. Intravenous carnitine (200–500 mg/kg/day) and biotin (10–20 mg/day) can be useful. If there is hyperammonemia, carglumic acid (50–100 mg/kg/day) should be started; non-responders may need extracorporeal detoxification.[5]

After stabilization, a low-protein diet supplemented with an amino acid mixture devoid of valine and isoleucine can be started.[5] Catabolism should be avoided. Carnitine supplementation (50–100 mg/kg/day) and metronidazole 10–20 mg/kg/day for 10 days per month should be considered. In anemic infants, erythropoietin can be considered but is often not effective.[101] Current evidence favors liver transplant as the eventual, effective treatment.[102] Figure 8 shows the key principles of management.

Monitoring: Patients should be periodically monitored for organic acids in urine, amino acid profile in blood, acylcarnitine profile including free carnitine in dried blood, ammonia, lactate, and blood leukocyte counts.[5,10,57]

Outcome: Guarded.[103]

1D. Isovaleric Academia (IVA)[104–106]

Isovaleric academia is an AR disorder due to the deficiency of isovaleryl-CoA dehydrogenase (IVD) required in leucine metabolism.[11] A study in Germany showed C.932C>T mutation in 47% alleles.[107]

Clinical symptoms: Isovaleric academia usually presents in the neonatal period with signs of OA.[108] The characteristic odor of sweaty feet makes it relatively easy to diagnose. A few patients present later in infancy with lethargy, vomiting, and coma; the clinical features mimic diabetes ketoacidosis and pancreatitis.

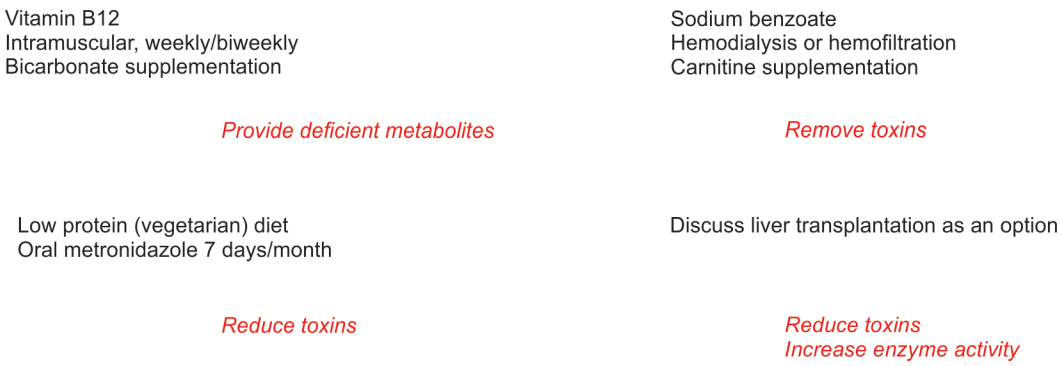


Fig. 8: Management principles to treat propionic acidemia

Diagnosis: Detectable isovaleryl glycine and 3-hydroxyisovaleric acid in urine by GC-MS, and increased isovaleryl-2-methylbutyryl carnitine (C5) in plasma suggests IVA.[108] Confirmation of diagnosis requires deficient activity of isovaleryl-CoA dehydrogenase in skin fibroblasts.[109] Genetic diagnosis of IVA can be done in the NBS; the aforementioned German study showed that NBS identified 13/19 patients with IVA.[107]

Management: Patients suspected to have IVA should be stated on a low natural protein diet, which can be cautiously increased per tolerance and growth, development, metabolic control, and plasma levels of essential amino acids. Leucine is to be avoided in amino acid mixtures. Glycine 15–250 mg/kg can be started to facilitate the excretion of isovaleryl glycine in severe IVA.[11] L- Carnitine (100–300 mg/kg/day) in three divided doses can be useful.[11,110]

Prognosis: Patients with IVA have better outcomes than those with MMA and PA.[111]

1E. 3-methylcrotonylglycinuria (3-MCG)[112–114]

3-MCG is seen in about 1 in 50,000 neonates and is the most frequently seen OA. There are two subtypes:

Biotin-resistant 3-MCG: It is caused by a deficiency of 3-methylcrotonyl-CoA carboxylase (MCC), a biotin-dependent but biotin-resistant carboxylase that is required for catabolism of leucine.[5] It converts 3-methylcrotonyl-CoA to 3-methylglutaconyl-CoA.

Biotin-responsive 3-MCG: This condition can result from a deficiency of holocarboxylase synthetase or biotinidase, leading to multiple carboxylase deficiency.[115] Further characterization of this enzyme is needed; the severity of enzyme deficiency does not correlate with disease manifestations. There is a possibility that multiple isoforms exist.[116] It may have a low penetrance or may need other genetic or environmental stimuli to manifest a phenotype.

Clinical symptoms: Most children with 3-MCG in NBS remain asymptomatic.[113] There have been rare instances of symptoms in the neonatal period with seizures and hypotonia. Most patients present between 6 months to 3 years after birth with developmental delay, FTT, hypoglycemia, and acidosis. Some become symptomatic during periods of increased catabolism.

Diagnosis: Patients with 3-MCG show increased urinary levels of 3-hydroxy isovaleric acid and 3-methyl crotonyl glycine, and low plasma levels of total and free plasma carnitine.[117] Plasma levels of 3-hydroxy isovaleryl carnitine concentration are increased. Due to secondary carboxylase deficiency, urinary levels of 3-methyl citrate, propionic acid, and propionyl carnitine are increased. Molecular testing in leukocytes or fibroblasts can confirm the diagnosis.

Management: Biotin (5–10 mg/day) should be started.[118] If free carnitine is deficient, L-carnitine is supplemented. Most patients respond to carnitine supplementation.[119] Symptomatic patients respond to a diet with low natural protein diet. Leucine restriction is not required.

Prognosis

3-MCG has a good prognosis for survival and neurodevelopment.

1F. 3-Methylglutaconic Aciduria (MGA)[120–122]

There are five types of 3-MGA.[12]

Type I: A rare AR condition caused by 3-methylglutaconyl-CoA hydratase deficiency.[123] This enzyme converts 3-methylglutaconyl-CoA to 3-hydroxy-3-methylglutaryl-CoA, which is essential in leucine degradation. These patients show high levels of 3-methylglutaconic acid, 3-methyl glutaric acid, and 3-hydroxyisovaleric acid in urine.

Clinical symptoms: The clinical spectrum ranges from asymptomatic newborns identified in NBS to adults with advanced neurodegeneration.[124]

Management: Restricted leucine and L-carnitine supplementation.[124]

Prognosis: Guarded.

Type II: This is an X-linked disorder, also called Barth syndrome.[125] The condition is caused by mutations in the tafazzin (TAZ) gene.[126] Tafazzin acetyltransferase activity is responsible for remodeling cardiolipin, which is a part of the inner mitochondrial membrane and is required for the respiratory chain activity.[127]

Clinical symptoms: Barth syndrome presents with cardiomyopathy, skeletal myopathy, FTT, and neutropenia. The presentation may vary within a single family.[128]

Many patients show absolute neutrophil counts less than 1.5×10^9/L.[129] About 50% may show chronic severe neutropenia with multiple counts below 0.5×10^9/L. Neutropenia can be chronic and severe, or cyclical with mathematically regular oscillations. Some patients also show monocytosis with absolute monocyte counts above 1×10^9/L. Recombinant granulocyte colony-stimulating factor (rG-CSF) can be effective. Some patients may also need antibiotic prophylaxis.

Diagnosis: Strong family history is a clue to diagnosis. Increased urine 3-MGA levels support but do not confirm the diagnosis.

Molecular testing is required. Tetralinoleoyl cardiolipin levels are low.[130]

Management: Barth syndrome is managed symptomatically, with particular emphasis on the treatment of cardiomyopathy and neutropenia.[128] ANCs usually rise upon rGSF administration.[131]

Type III: This is a rare AR disorder, also known as Costeff syndrome. The genetic cause is mutations in the outer mitochondrial membrane lipid metabolism regulator (OPA3) gene.[132]

Clinical symptoms: It involves early-onset optic atrophy and neurologic symptoms like cognitive impairment, and is therefore frequently named as the neuro-ophthalmologic syndrome.[132,133]

Diagnosis: Most patients show intermittent elevations in urine 3-MGA levels. Molecular testing confirms the diagnosis.[133,134]

Management: Current treatment is limited to supportive measures.

Prognosis: Guarded.

Type IV: This is a heterogeneous group where no genetic defect has been consistently identified so far. Some patients have mitochondrial respiratory chain disorders.[135]

Clinical symptoms: There might be four clinical subgroups, encephalomyopathic, hepatocerebral, cardiomyopathic, and myopathic. However, the cohorts have been small, and further study is needed. Cataracts, epilepsy, and neutropenia may be seen in patients who have variants in the caseinolytic peptidase B homolog (CPLB).[135]

Management: Current treatment is limited to supportive measures.

Prognosis: Guarded.

Type V: This disorder has been associated with mutations in the DnaJ heat shock protein family (Hsp 40) member C19 (DNAJC19),[136] which encodes a protein involved in importing other proteins into the mitochondria.[137]

Clinical symptoms: The condition is seen more frequently in the Canadian Dariusleut-Hutterite ethnicity.[138] Patients frequently have cerebellar ataxia, testicular dysgenesis, cardiomyopathy, and growth failure.[12]

Management: Current treatment is limited to supportive measures.

Prognosis: Guarded.

2. Multiple Carboxylase Deficiency

2A. Holocarboxylase Synthetase Deficiency (HLCS)[112–114]

Holocarboxylase synthetase (HLCS) deficiency is a multiple carboxylase deficiency, where the activity of several biotin-dependent enzymes is impaired.[15]

Genetics: It is an AR inherited disorder associated with nearly 50 known mutations in this gene.[139] The overall incidence of this disorder has been estimated as 1 in 87,000.[15,140]

Holocarboxylase synthetase is the only protein biotin ligase in the human proteome.[141] It plays several important roles where it:

- Activates many biotin-dependent carboxylases needed for macronutrient metabolism.[142]
- Acts as an essential component of several multiprotein complexes in chromatin that regulate many target genes and also contribute to genome stability.[143]
- Forms a positive feedback regulatory cycle as it has a biotin-binding domain and thus carries a biotin reservoir. Biotin then activates its own expression by binding its 3 promoters, named P1, P2, and P3.[144,145]
- Alters gene expression by promoting biotin binding to lysine (K) residues in histones H2A, H3, and H4.[146,147] H3 biotinylation on its K9 and K18 has been studied in detail.[146]

Clinical features: HCS deficiency typically presents in early infancy.[15] There may be feeding difficulties, dyspnea, skin rash, alopecia, and/or lethargy.[140] Many patients show delayed development, seizures, and altered sensorium.

Treatment: Lifelong biotin supplementation can attenuate clinical manifestations.[15]

Prognosis: Guarded.[15]

2B. Biotinidase (BTD) Deficiency

Biotinidase (BTD) is a ubiquitously expressed enzyme essential for recycling the vitamin biotin, a water-soluble cofactor necessary for the function of several carboxylases.[148] Biotinidase expresses biotinyl-hydrolase and biotinyl-transferase activities.[149] It is a monomeric sialylated glycoprotein with multiple isoforms.[150] Deficiency of BTD activity with secondary disruption of relevant carboxylases can disrupt several pathways in amino acid, carbohydrate, and lipid metabolism.[148]

The incidence of BTD deficiency is 1 in 137,401 for profound BTD deficiency, 1 in 109,921 for partial, and 1 in 61,067 for the combined incidence of profound and partial BTD deficiency.[16,151] The incidence rises with consanguinity. It may occur more frequently in Latinos, but not in African Americans.[151,152] Carriers are seen in the general population at the rates of 1 in 120.[151]

Genetics: Biotinidase deficiency is an AR condition.[153] The BTD gene consists of 4 exons and 3 introns. The gene shows variants that are benign, likely benign, those of uncertain significance, and others that are likely pathogenic, or pathogenic.[154] There might be small intragenic deletions/insertions; and missense, nonsense, and splice site variants. Exon or whole-gene deletions/duplications are not detected frequently.[153] Pathogenic c.1330G>C (p.Asp444His) and c.1368A>C (p.Gln456His) variants have been noted in individuals of Amish ancestry.[155]

Genotype–phenotype correlations are not well established. A report from Turkey showed pathogenic null variants that led to profound enzyme deficiency had hearing loss, but those who had some residual enzyme activity did not.[156] Compound heterozygotes for the p.Asp444His pathogenic variant and a pathogenic variant that results in profound BTD deficiency are expected to have approximately 20–25% of mean normal serum BTD enzyme activity.[157] Homozygotes for the p.Asp444His variant may have about half of normal serum BTD activity; they present clinically like heterozygotes for profound BTD deficiency.[158]

Clinical features: Timely diagnosis in newborn screening and biotin therapy can prevent clinical illness.[153] Early clinical features may include seizures, developmental delay, rash and alopecia, optic atrophy, hearing loss, and respiratory problems. These infants may also show hyperventilation, laryngeal stridor, and central apnea.[153,159] Most respond to biotin therapy.

Untreated profound BTD deficiency (<10% of normal serum BTD activity) may cause optic atrophy, hypotonia, seizures, hair loss, and skin rash.[153] Cellular immunodeficiency may increase the risk of recurrent viral or fungal infections.[160] Partial BTD deficiency (10–30% of normal serum BTD activity) may become symptomatic

during stress such as during infections. Brain imaging may show cerebral atrophy and ventricular dilatation.[153] These abnormalities and feeding issues, skin lesions, and respiratory issues may resolve with biotin therapy, but optic atrophy, hearing loss, and developmental delay may not reverse completely.[153]

Treatment: Infants with profound and partial BTD deficiency should be treated with free (not protein-bound) biotin, administered orally, for life. Patients with profound deficiency should receive 5–10 mg biotin orally every day, whereas those with partial deficiency may improve with 2.5–10 mg daily. Biotin therapy can completely prevent or reduce the severity of symptoms. Metabolic and hemodynamic support are important. Supportive developmental therapy, and evaluation of vision and hearing are important.

Prenatal diagnosis: If the pathogenic variants in the family are known, preimplantation or fetal genetic testing can be done. In a family with known pathogenic variants causing BTD deficiency, amniocentesis or chorionic villus sampling should be considered to facilitate institution of treatment at birth. If prenatal testing was not possible, a newborn with an older sibling with known BTD deficiency should receive biotin pending definitive results of screening.[161]

Once a molecular diagnosis has been established in the proband, genetic testing of parents can confirm if they are heterozygotes. If a pathogenic variant is detected in only one parent and parental identity testing has been confirmed, there are two possibilities: (a) one of the pathogenic variants occurred as a de novo event in the proband or during the postzygotic period in a mosaic parent; (b) uniparental isodisomy for the parental chromosome with the pathogenic variant resulted in homozygosity for the pathogenic variant in the proband.[155]

Prognosis: The detection of presymptomatic infants has improved the outcomes. A few deaths are still noted in individuals with severe metabolic compromise.[153]

3. Cerebral Organic Acidemias

3A. Glutaric Acidemia type 1 (GA1)

Glutaric acidemia type 1 (GA1) is an AR disorder seen 1 in 30,000–100,000 neonates.[162] It is seen more frequently in Scandinavian countries; in native residents of North America, particularly in northeast Manitoba, northwestern Ontario; and in the Amish in Pennsylvania.[93]

Genetics: GA1 is caused by deficiency of glutaryl-CoA dehydrogenase (GCDH), a riboflavin-dependent mitochondrial enzyme that converts glutaryl-CoA to crotonyl-CoA in the catabolic pathway of lysine, hydroxylysine, and tryptophan.[163]

Clinical symptoms: The presentation is quite variable. Most patients present after the neonatal period and the clinical features do not always correlate with the biochemical genotype; 25% do not manifest notable clinical features. After infancy, nearly 75% patients present during/after infections with ketoacidosis, hyperammonemia, hypoglycemia, and encephalopathy. There may be feeding difficulties due to orofacial dyskinesia. Some show an irreversible dystonic movement disorder with preserved cognitive function,[18] and may be misdiagnosed as cerebral palsy.

Some infants with GA1 are born with macrocephaly.[93] These patients are more likely to develop movement disorders with spasms, jerking, rigidity, or decreased muscle tone. Others are born with microcephaly but then show rapid head growth during infancy. Due to the increased fragility of bridging veins stretched because of cerebral atrophy, 20–30% of these children may present with acute subdural hemorrhage or chronic subdural effusions.[164] These may be wrongly ascribed to child abuse or shaken baby syndrome.

Some patients present with late-onset disease after 6 years of age with nonspecific neurologic abnormalities, vertigo, dementia, and ataxia.[165] Some may manifest with stroke due to acute symmetric striatal necrosis.[166] Cases with injury in the putamen may show developmental arrest. Autopsy findings suggest that brain injury may be related to the efficiency of organic acid clearance.

Seizures may be seen in 20% of children. Insomnia, hyperthermia, hyperhidrosis, and anorexia may be seen in some of them.[31] Some may show decline in renal functions over time.

Diagnosis:[165,167] Prenatal testing by amniotic fluid or chorionic villous sampling can be helpful.[46] After birth, infants with GA1 usually show normal blood pH and levels of glucose and ammonia. There are increased urinary levels of glutaric acid and 3-hydroxyglutaric acid, and plasma glutarylcarnitine (C5DC) along with decreased carnitine.[93] It may present in a (a) classic presentation with elevated glutamate metabolites in blood and urine, and (b) low-excretor variant with normal or very minimally elevated levels of glutamate metabolites.[93] Confirmation requires identification of pathogenic mutations and deficient GCDH activity in fibroblasts or leucocytes.[165] Neuroimaging can provide helpful clues to diagnosis (Fig. 9).

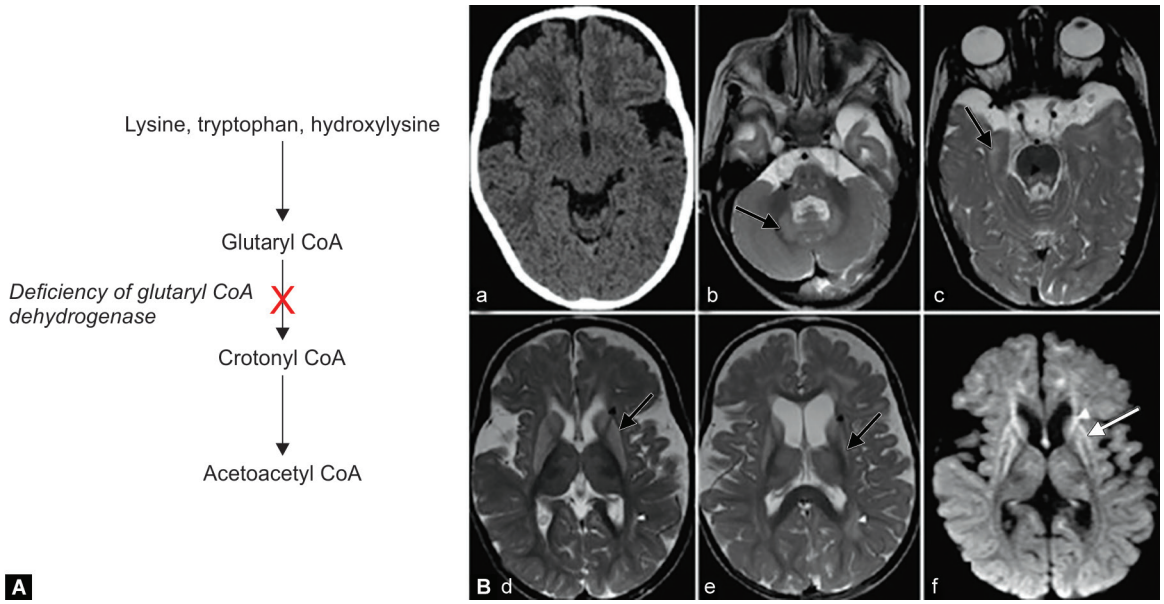
Management:[168] Early treatment reduces the risk of neurologic damage.[93] A low-protein diet should be started according to the requirement for growth and then increased gradually depending on the tolerance, growth development, plasma levels of essential amino acids. Tryptophan and lysine should not be introduced until 6 years of age. Isoleucine and valine to be given as needed.[18] These dietary restrictions should be managed under the supervision of a metabolic consultant.

L-carnitine (100–200 mg/kg/day intravenously, or 100–300 mg/kg/day orally divided in 3 doses) along with riboflavin (100-300 mg/day) may be started as it is a cofactor of the GCDH.[169] For dystonia, baclofen or valproic acid can help.[18]

Untreated patients with infections or fever after vaccination or surgery are at risk of developing an acute encephalopathic crisis.[165] Murine models of GA1 show depletion of glutamate and gamma-aminobutyric acid (GABA) in the brain, which worsens during acute crises. Preservation of these metabolites might help in improvement.[18]

Prognosis:[163,170] Patients with GA1, if diagnosed through NBS, can develop normally with timely initiation of *L*-carnitine supplementation and a low-protein diet. Metabolic decompensation should be prevented/treated aggressively in intercurrent sicknesses.[171] Those who remain undiagnosed and hence untreated can develop developmental delay with cerebral atrophy and pyramidal tract signs.[172] Magnetic resonance imaging show extracerebral fluid collections and atrophy in the frontotemporal areas and diffuse hypodensities in the white matter.[173] These patients may improve with dietary therapy, although some work still needs to be done.[18,174]

Early initiation of special formula and dietary modifications can be effective.[93] In a cohort of 168 patients who were diagnosed by NBS, and given special formula and dietary changes along with emergency treatment, only 7% showed striatal degeneration as compared with 47% of those who were not offered special formula.



Figs 9A and B: (A) Metabolic defect in glutaric aciduria type 1; (B) MRI findings in glutaric aciduria type 1. In addition to macrocephaly includes characteristic bilateral small anterior poles of temporal lobes with poor opercularization and widened sylvian fissure, subdural collections, and basal ganglia lesions

Nearly, 90% of those who were not offered NBS and dietary changes showed striatal changes.[163]

3B. N-Aspartoacylase Deficiency (Canavan Disease)

Canavan disease is an AR genetic neurological disorder characterized by the spongy degeneration of the white matter in the brain.[175] It is seen more frequently in the Ashkenazi Jewish population.[176]

Genetics: Loss-of-function mutations in the aspartoacylase (ASPA) gene result in accumulation of *N*-acetyl-L-aspartic acid (NAA).[177] Aspartoacylase is normally expressed in oligodendrocytes for myelin synthesis; some of the ongoing studies suggest that high levels of NAA may cause oxidative damage to the glia in the pathogenesis of this condition.[178,179]

Clinical features: Most patients show clinical features by 3-5 months after birth.[180] These include macrocephaly, irritability, feeding difficulties, hypotonia, delayed motor milestones, visual impairment, and seizures. Most patients show progressive brain atrophy.[180,181]

Treatment: Management is limited to supportive measures.[182]

Prognosis: The prognosis for Canavan disease is poor; most patients die before 10 years of age.[181] The juvenile form is relatively mild and may be seen in the later 1st and 2nd decades.[174]

3C. 2-Hydroxyglutaric Acidurias

2-hydroxyglutaric acidurias are rare, progressive neurodegenerative disorders.[183] These include (a) D-2-hydroxyglutaric aciduria (D-2-HGA); (b) L-2-hydroxyglutaric aciduria (L-2-HGA); and (c) combined D,L-2-hydroxyglutaric aciduria (D,L-2-HGA).[184] Only a few hundred cases have been reported.[183] These conditions are associated with accumulation of toxic metabolites that can cause neuronal apoptosis.[185]

Genetics: Loss-of-function mutations in D-2-hydroxyglutarate dehydrogenase (D2HGDH) and isocitrate dehydrogenase-2 (IDH2) genes, which encode mitochondrial enzymes that metabolize D-2-HG acid, result in types I and II D-2-HG aciduria.[186] L-2-HG aciduria is associated with mutations in the L-2-hydroxyglutarate dehydrogenase (L2HGDH) gene.[187] Patients with combined D,L-2-HGA had mutations in the solute carrier family 25-member 1 (SLC25A1) gene, which can alter the function of related enzymes. D-2-HGA type I, L-2-HGA, and combined D,L-2-HGA follow AR inheritance, but D-2-HGA type II, which involves mutations in the IDH2 genes, follows an autosomal dominant (AD) pattern.[184]

Clinical features: D-2-HGA manifests with delayed development, seizures, hypotonia, and visual dysfunction.[188] There may be two subtypes of this disorder, I and II.[184] Type II tends to begin earlier, has more severe neurological dysfunction, and may be associated with cardiomyopathy.[189] L-2-HGA is marked by macrocephaly, developmental delay, seizures, and cerebellar dysfunction with ataxia.[183] Clinical manifestations usually begin during early infancy and worsen during adolescence. Combined D,L-2-HGA causes manifests during early infancy with hypotonia, seizures, and difficulties in breathing and feeding.

Treatment: Management is limited to supportive measures.

Prognosis: The condition is usually lethal by early childhood.[183]

Conclusion

In this review, we have summarized currently available information on OAs. We need to develop cost-effective but sufficiently comprehensive neonatal screening programs for various regions, understand the genetics, methods for early clinical identification, and treatment.

Orcid

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

References

1. Ramsay J, Morton J, Norris M, et al. Organic acid disorders. Ann Transl Med 2018;6(24):472. DOI: 10.21037/atm.2018.12.39.

2. Dimitrov B, Molema F, Williams M, et al. Organic acidurias: Major gaps, new challenges, and a yet unfulfilled promise. J Inherit Metab Dis 2021;44(1):9–21. DOI: 10.1002/jimd.12254.
3. Md AN. Neonatal Presentations of Metabolic Disorders. Neoreviews 2020;21(10):e649–e662. DOI: 10.1542/neo.21-10-e649.
4. Vaidyanathan K, Narayanan MP, Vasudevan DM. Organic acidurias: An updated review. Indian J Clin Biochem 2011;26(4):319–325. DOI: 10.1007/s12291-011-0134-2.
5. Baumgartner MR, Horster F, Dionisi-Vici C, et al. Proposed guidelines for the diagnosis and management of methylmalonic and propionic acidemia. Orphanet J Rare Dis 2014;9:130. DOI: 10.1186/s13023-014-0130-8.
6. Villani GR, Gallo G, Scolamiero E, et al. Classical organic acidurias: Diagnosis and pathogenesis. Clin Exp Med 2017;17(3):305–323. DOI: 10.1007/s10238-016-0435-0.
7. Boy N, Muhlhausen C, Maier EM, et al. Proposed recommendations for diagnosing and managing individuals with glutaric aciduria type I: second revision. J Inherit Metab Dis 2017;40(1):75–101. DOI: 10.1007/s10545-016-9999-9.
8. Ogier de Baulny H, Saudubray JM. Branched-chain organic acidurias. Semin Neonatol 2002;7(1):65–74. DOI: 10.1053/siny.2001.0087.
9. Manoli I, Sloan JL, Venditti CP. Isolated Methylmalonic Acidemia. 2022. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.
10. Shchelochkov OA, Carrillo N, Venditti CP. Propionic Acidemia. 2016. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.
11. Vockley J, Ensenauer R. Isovaleric acidemia: New aspects of genetic and phenotypic heterogeneity. Am J Med Genet C Semin Med Genet 2006;142C(2):95–103. DOI: 10.1002/ajmg.c.30089.
12. Wortmann SB, Kluijtmans LA, Engelke UF, et al. The 3-methylglutaconic acidurias: what's new? J Inherit Metab Dis 2012;35(1):13–22. DOI: 10.1007/s10545-010-9210-7.
13. Gallardo ME, Desviat LR, Rodriguez JM, et al. The molecular basis of 3-methylcrotonylglycinuria, a disorder of leucine catabolism. Am J Hum Genet 2001;68(2):334–346. DOI: 10.1086/318202.
14. Wolf B, Hsia YE, Sweetman L, et al. Multiple carboxylase deficiency: Clinical and biochemical improvement following neonatal biotin treatment. Pediatrics 1981;68(1):113–118. PMID: 6787561.
15. Donti TR, Blackburn PR, Atwal PS. Holocarboxylase synthetase deficiency pre and post newborn screening. Mol Genet Metab Rep 2016;7:40–44. DOI: 10.1016/j.ymgmr.2016.03.007.
16. Wolf B. Biotinidase deficiency: If you have to have an inherited metabolic disease, this is the one to have. Genet Med 2012;14(6):565–575. DOI: 10.1038/gim.2011.6.
17. Reddy N, Calloni SF, Vernon HJ, et al. Neuroimaging Findings of Organic Acidemias and Aminoacidopathies. Radiographics 2018;38(3):912–931. DOI: 10.1148/rg.2018170042.
18. Kolker S, Christensen E, Leonard JV, et al. Diagnosis and management of glutaric aciduria type I–revised recommendations. J Inherit Metab Dis 2011;34(3):677–694. DOI: 10.1007/s10545-011-9289-5.
19. Wijayasinghe YS, Pavlovsky AG, Viola RE. Aspartoacylase catalytic deficiency as the cause of Canavan disease: A structural perspective. Biochemistry 2014;53(30):4970–4978. DOI: 10.1021/bi500719k.
20. Kim YG, Lee S, Kwon OS, et al. Redox-switch modulation of human SSADH by dynamic catalytic loop. EMBO J 2009;28(7):959–968. DOI: 10.1038/emboj.2009.40.
21. Didiasova M, Banning A, Brennenstuhl H, et al. Succinic semialdehyde dehydrogenase deficiency: An update. Cells 2020;9(2). DOI: 10.3390/cells9020477.
22. Seashore MR. The organic acidemias: An overview 2009. In: GeneReviews [Internet]. Seattle: University of Washington, Seattle.
23. Wajner M. Neurological manifestations of organic acidurias. Nat Rev Neurol 2019;15(5):253–271. DOI: 10.1038/s41582-019-0161-9.
24. Chapman KA, Gropman A, MacLeod E, et al. Acute management of propionic acidemia. Mol Genet Metab 2012;105(1):16–25. DOI: 10.1016/j.ymgme.2011.09.026.
25. Byers SL, Ficicioglu C. Infant with cardiomyopathy: When to suspect inborn errors of metabolism? World J Cardiol 2014;6(11):1149–55. DOI: 10.4330/wjc.v6.i11.1149.
26. Fraser JL, Venditti CP. Methylmalonic and propionic acidemias: Clinical management update. Curr Opin Pediatr 2016;28(6):682–93. DOI: 10.1097/MOP.0000000000000422.
27. Therrell BL, Padilla CD, Loeber JG, et al. Current status of newborn screening worldwide: 2015. Semin Perinatol 2015;39(3):171–87. DOI: 10.1053/j.semperi.2015.03.002.
28. Watson MS, Lloyd-Puryear MA, Howell RR. The progress and future of US newborn screening. Int J Neonatal Screen 2022;18;8(3):41. DOI: 10.3390/ijns8030041.
29. Tuncel AT, Boy N, Morath MA, et al. Organic acidurias in adults: Late complications and management. J Inherit Metab Dis 2018;41(5):765–776. DOI: 10.1007/s10545-017-0135-2.
30. Mosleh T, Dey SK, Mannan MA. A Case of Organic Acidemia: Are Physicians Aware Enough? Euroasian J Hepatogastroenterol 2016;6(1):89–90. DOI: 10.5005/jp-journals-10018-1175.
31. Afzal RM, Lund AM, Skovby F. The impact of consanguinity on the frequency of inborn errors of metabolism. Mol Genet Metab Rep 2018;15:6–10. DOI: 10.1016/j.ymgmr.2017.11.004.
32. Sudo Y, Sasaki A, Wakabayashi T, et al. A novel ETFB mutation in a patient with glutaric aciduria type II. Hum Genome Var 2015;1:15016. DOI: 10.1038/hgv.2015.16.
33. Balakrishnan U. Inborn errors of metabolism-approach to diagnosis and management in neonates. Indian J Pediatr 2021;88(7):679–689. DOI: 10.1007/s12098-021-03759-9.
34. Haberle J, Chakrapani A, Ah Mew N, et al. Hyperammonaemia in classic organic acidaemias: A review of the literature and two case histories. Orphanet J Rare Dis 2018;13(1):219. DOI: 10.1186/s13023-018-0963-7.
35. Kelley RI, Cheatham JP, Clark BJ, et al. X-linked dilated cardiomyopathy with neutropenia, growth retardation, and 3-methylglutaconic aciduria. J Pediatr 1991;119(5):738–747. DOI: 10.1016/s0022-3476(05)80289-6.
36. Schillaci LP, DeBrosse SD, McCandless SE. Inborn errors of metabolism with acidosis: Organic acidemias and defects of pyruvate and ketone body metabolism. Pediatr Clin North Am 2018;65(2):209–230. DOI: 10.1016/j.pcl.2017.11.003.
37. Fu X, Iga M, Kimura M, et al. Simplified screening for organic acidemia using GC/MS and dried urine filter paper: A study on neonatal mass screening. Early Hum Dev 2000;58(1):41–55. DOI: 10.1016/s0378-3782(00)00053-0.
38. Wajner M, Sitta A, Kayser A, et al. Screening for organic acidurias and aminoacidopathies in high-risk Brazilian patients: Eleven-year experience of a reference center. Genet Mol Biol 2019;42(1 suppl 1):178–185. DOI: 10.1590/1678-4685-GMB-2018-0105.
39. Webb BD, Nowinski SM, Solmonson A, et al. Recessive pathogenic variants in MCAT cause combined oxidative phosphorylation deficiency. Elife 2023;12. DOI: 10.7554/eLife12:e68047.
40. Van Cauter S, Severino M, Ammendola R, et al. Bilateral lesions of the basal ganglia and thalami (central grey matter)-pictorial review. Neuroradiology 2020;62(12):1565–1605. DOI: 10.1007/s00234-020-02511-y.
41. Brismar J, Ozand PT. CT and MR of the brain in the diagnosis of organic acidemias. Experiences from 107 patients. Brain Dev 1994;16 Suppl:104–124. DOI: 10.1016/0387-7604(94)90103-1.
42. Maliia MD, Donos C, Barborica A, et al. Functional mapping and effective connectivity of the human operculum. Cortex 2018;109:303–321. DOI: 10.1016/j.cortex.2018.08.024.
43. Gallagher RC, Pollard L, Scott AI, et al. Laboratory analysis of organic acids, 2018 update: A technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2018;20(7):683–691. DOI: 10.1038/gim.2018.45.
44. Alberola TM, Bautista-Llacer R, Vendrell X, et al. Case report: Birth of healthy twins after preimplantation genetic diagnosis of propionic acidemia. J Assist Reprod Genet 2011;28(3):211–216. DOI: 10.1007/s10815-010-9514-4.

45. Ding S, Liang L, Qiu W, et al. Prenatal Diagnosis of Isovaleric Acidemia From Amniotic Fluid Using Genetic and Biochemical Approaches. Front Genet 2022;13:898860. DOI: 10.3389/fgene.2022.898860.
46. Xiao B, Qiu W, Ye J, et al. Prenatal Diagnosis of Glutaric Acidemia I Based on Amniotic Fluid Samples in 42 Families Using Genetic and Biochemical Approaches. Front Genet 2020;11:496. DOI: 10.3389/fgene.2020.00496.
47. Tanacan A, Gurbuz BB, Aydin E, et al. Prenatal Diagnosis of Organic Acidemias at a Tertiary Center. Balkan J Med Genet 2019;22(1):29–34. DOI: 10.2478/bjmg-2019-0003.
48. Tiwana SK, Rascati KL, Park H. Cost-effectiveness of expanded newborn screening in Texas. Value Health 2012;15(5):613–621. DOI: 10.1016/j.jval.2012.02.007.
49. Baker PR 2nd. Recognizing and Managing a Metabolic Crisis. Pediatr Clin North Am 2023;70(5):979–993. DOI: 10.1016/j.pcl.2023.05.009.
50. Guerrero RB, Salazar D, Tanpaiboon P. Laboratory diagnostic approaches in metabolic disorders. Ann Transl Med 2018;6(24):470. DOI: 10.21037/atm.2018.11.05.
51. Maiorana A, Lepri FR, Novelli A, et al. Hypoglycaemia metabolic gene panel testing. Front Endocrinol (Lausanne) 2022;13:826167. DOI: 10.3389/fendo.2022.826167.
52. Seifter JL. Anion-gap metabolic acidemia: Case-based analyses. Eur J Clin Nutr 2020;74(Suppl 1):83–86. DOI: 10.1038/s41430-020-0685-5.
53. Shakerdi LA, Gillman B, Corcoran E, et al. Organic aciduria disorders in pregnancy: An overview of metabolic considerations. Metabolites 2023;13(4). DOI: 10.3390/metabo13040518.
54. Chakrapani A, Valayannopoulos V, Segarra NG, et al. Effect of carglumic acid with or without ammonia scavengers on hyperammonaemia in acute decompensation episodes of organic acidurias. Orphanet J Rare Dis 2018;13(1):97. DOI: 10.1186/s13023-018-0840-4.
55. Levrat V, Forest I, Fouilhoux A, et al. Carglumic acid: An additional therapy in the treatment of organic acidurias with hyperammonemia? Orphanet J Rare Dis 2008;3:2. DOI: 10.1186/1750-1172-3-2.
56. Gugelmo G, Lenzini L, Francini-Pesenti F, et al. Anthropometrics, Dietary Intake and Body Composition in Urea Cycle Disorders and Branched Chain Organic Acidemias: A Case Study of 18 Adults on Low-Protein Diets. Nutrients 2022;14(3). DOI: 10.3390/nu14030467.
57. Forny P, Horster F, Ballhausen D, et al. Guidelines for the diagnosis and management of methylmalonic acidaemia and propionic acidaemia: First revision. J Inherit Metab Dis 2021;44(3):566–592. DOI: 10.1002/jimd.12370.
58. Mobarak A, Dawoud H, Nofal H, et al. Clinical course and nutritional management of propionic and methylmalonic acidemias. J Nutr Metab 2020;2020:8489707. DOI: 10.1155/2020/8489707.
59. de Lonlay P, Valayannopoulos V, Arnoux JB, et al. [Diagnostic and therapeutic management of inherited metabolic diseases in emergency and intensive care unit]. Arch Pediatr 2010;17(6):947–948. DOI: 10.1016/S0929-693X(10)70192-5.
60. Sperl W. Diagnosis and therapy of organic acidurias. Padiatr Padol 1993;28(1):3–8. PMID: 8446425.
61. Blackburn PR, Gass JM, Vairo FPE, et al. Maple syrup urine disease: Mechanisms and management. Appl Clin Genet 2017;10:57–66. DOI: 10.2147/TACG.S125962.
62. Campanholi DRR, Margutti AVB, Silva WA Jr., et al. Molecular basis of various forms of maple syrup urine disease in Chilean patients. Mol Genet Genomic Med 2021;9(5):e1616. DOI: 10.1002/mgg3.1616.
63. Strauss KA, Puffenberger EG, Carson VJ. Maple Syrup Urine Disease 2020. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.
64. Puffenberger EG. Genetic heritage of the Old Order Mennonites of southeastern Pennsylvania. Am J Med Genet C Semin Med Genet 2003;121C(1):18–31. DOI: 10.1002/ajmg.c.20003.
65. Love-Gregory LD, Grasela J, Hillman RE, et al. Evidence of common ancestry for the maple syrup urine disease (MSUD) Y438N allele in non-Mennonite MSUD patients. Mol Genet Metab 2002;75(1):79–90. DOI: 10.1006/mgme.2001.3264.
66. Schadewaldt P, Bodner-Leidecker A, Hammen HW, et al. Significance of L-alloisoleucine in plasma for diagnosis of maple syrup urine disease. Clin Chem 1999;45(10):1734–1740. PMID: 10508118.
67. Hassan SA, Gupta V. Maple Syrup Urine Disease. 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
68. Ali EZ, Ngu LH. Fourteen new mutations of BCKDHA, BCKDHB and DBT genes associated with maple syrup urine disease (MSUD) in Malaysian population. Mol Genet Metab Rep 2018;17:22–30. DOI: 10.1016/j.ymgmr.2018.08.006.
69. Podebrad F, Heil M, Reichert S, et al. 4,5-dimethyl-3-hydroxy-2[5H]-furanone (sotolone–the odour of maple syrup urine disease. J Inherit Metab Dis. 1999;22(2):107–114. DOI: 10.1023/a:1005433516026.
70. Lin YT, Cai YN, Ting TH, et al. Diagnosis of an intermediate case of maple syrup urine disease: A case report. World J Clin Cases 2023;11(5):1077–1085. DOI: 10.12998/wjcc.v11.i5.1077.
71. Rawal S, Faghfoury H, Krings T. MRI findings of adult maple syrup urine disease exacerbation. Can J Neurol Sci 2013;40(2):259–262. DOI: 10.1017/s0317167100013858.
72. Chuang DT, Ku LS, Cox RP. Thiamin-responsive maple-syrup-urine disease: Decreased affinity of the mutant branched-chain alpha-keto acid dehydrogenase for alpha-ketoisovalerate and thiamin pyrophosphate. Proc Natl Acad Sci U S A 1982;79(10):3300–3304. DOI: 10.1073/pnas.79.10.3300.
73. Quinonez SC, Seeley AH, Seeterlin M, et al. Newborn screening for dihydrolipoamide dehydrogenase deficiency: Citrulline as a useful analyte. Mol Genet Metab Rep 2014;1:345–349. DOI: 10.1016/j.ymgmr.2014.07.007.
74. Piri-Moghadam H, Miller A, Pronger D, et al. A rapid LC-MS/MS assay for detection and monitoring of underivatized branched-chain amino acids in maple syrup urine disease. J Mass Spectrom Adv Clin Lab 2022;24:107–117. DOI: 10.1016/j.jmsacl.2022.04.003.
75. Frazier DM, Allgeier C, Homer C, et al. Nutrition management guideline for maple syrup urine disease: an evidence - and consensus-based approach. Mol Genet Metab 2014;112(3):210–217. DOI: 10.1016/j.ymgme.2014.05.006.
76. Strauss KA, Wardley B, Robinson D, et al. Classical maple syrup urine disease and brain development: Principles of management and formula design. Mol Genet Metab 2010;99(4):333–345. DOI: 10.1016/j.ymgme.2009.12.007.
77. Puliyanda DP, Harmon WE, Peterschmitt MJ, et al. Utility of hemodialysis in maple syrup urine disease. Pediatr Nephrol 2002;17(4):239–242. DOI: 10.1007/s00467-001-0801-2.
78. Thimm E, Hadzik B, Hohn T. Continuous venovenous hemofiltration rapidly lowers toxic metabolites in a patient with MSUD and imminent cerebral herniation. Klin Padiatr 2010;222(4):264–265. DOI: 10.1055/s-0030-1247508.
79. Wendel U, Langenbeck U, Lombeck I, et al. Exchange transfusion in acute episodes of maple syrup urine disease. Studies on branched-chain amino and keto acids. Eur J Pediatr 1982;138(4):293–296. DOI: 10.1007/BF00442499.
80. Mazariegos GV, Morton DH, Sindhi R, et al. Liver transplantation for classical maple syrup urine disease: Long-term follow-up in 37 patients and comparative United Network for Organ Sharing experience. J Pediatr 2012;160(1):116–121 e1. DOI: 10.1016/j.jpeds.2011.06.033.
81. Shellmer DA, DeVito Dabbs A, Dew MA, et al. Cognitive and adaptive functioning after liver transplantation for maple syrup urine disease: A case series. Pediatr Transplant 2011;15(1):58–64. DOI: 10.1111/j.1399-3046.2010.01411.x.
82. Tchan M, Westbrook M, Wilcox G, et al. The management of pregnancy in maple syrup urine disease: experience with two patients. JIMD Rep 2013;10:113–117. DOI: 10.1007/8904_2013_212.
83. Pontoizeau C, Simon-Sola M, Gaborit C, et al. Neonatal gene therapy achieves sustained disease rescue of maple syrup urine disease in mice. Nat Commun 2022;13(1):3278. DOI: 10.1038/s41467-022-30880-w.
84. Kose M, Canda E, Kagnici M, et al. A Patient with MSUD: Acute management with sodium phenylacetate/sodium benzoate and

sodium phenylbutyrate. Case Rep Pediatr 2017;2017:1045031. DOI: 10.1155/2017/1045031.
85. Zhou X, Cui Y, Han J. Methylmalonic acidemia: Current status and research priorities. Intractable Rare Dis Res 2018;7(2):73–78. DOI: 10.5582/irdr.2018.01026.
86. Waisbren SE. Review of neuropsychological outcomes in isolated methylmalonic acidemia: Recommendations for assessing impact of treatments. Metab Brain Dis 2022;37(5):1317–1335. DOI: 10.1007/s11011-022-00954-1.
87. Sloan JL, Carrillo N, Adams D, et al. Disorders of Intracellular Cobalamin Metabolism. 2021. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.
88. Buers I, Pennekamp P, Nitschke Y, et al. Lmbrd1 expression is essential for the initiation of gastrulation. J Cell Mol Med 2016;20(8):1523–1533. DOI: 10.1111/jcmm.12844.
89. Paul C, Brady DM. Comparative Bioavailability and Utilization of Particular Forms of B(12) Supplements With Potential to Mitigate B(12)-related Genetic Polymorphisms. Integr Med (Encinitas) 2017;16(1):42–49. PMID: 28223907.
90. Takahashi-Iniguez T, Garcia-Hernandez E, Arreguin-Espinosa R, et al. Role of vitamin B12 on methylmalonyl-CoA mutase activity. J Zhejiang Univ Sci B 2012;13(6):423–437. DOI: 10.1631/jzus.B1100329.
91. Jin L, Han X, He F, et al. Prevalence of methylmalonic acidemia among newborns and the clinical-suspected population: A meta-analyse. J Matern Fetal Neonatal Med 2022;35(25):8952–8967. DOI: 10.1080/14767058.2021.2008351.
92. Jiang YZ, Sun LY. The Value of Liver Transplantation for Methylmalonic Acidemia. Front Pediatr 2019;7:87. DOI: 10.3389/fped.2019.00087.
93. Hedlund GL, Longo N, Pasquali M. Glutaric acidemia type 1. Am J Med Genet C Semin Med Genet 2006;142C(2):86-94. DOI: 10.1002/ajmg.c.30088.
94. Chen T, Gao Y, Zhang S, et al. Methylmalonic acidemia: Neurodevelopment and neuroimaging. Front Neurosci 2023;17:1110942. DOI: 10.3389/fnins.2023.1110942.
95. Al-Hamed MH, Imtiaz F, Al-Hassnan Z, et al. Spectrum of mutations underlying Propionic acidemia and further insight into a genotype-phenotype correlation for the common mutation in Saudi Arabia. Mol Genet Metab Rep 2019;18:22–29. DOI: 10.1016/j.ymgmr.2018.12.004.
96. Li Y, Wang M, Huang Z, et al. Novel compound heterozygous variants in the PCCB gene causing adult-onset propionic acidemia presenting with neuropsychiatric symptoms: A case report and literature review. BMC Med Genomics 2022;15(1):59. DOI: 10.1186/s12920-022-01202-2.
97. Yang X, Sakamoto O, Matsubara Y, et al. Mutation spectrum of the PCCA and PCCB genes in Japanese patients with propionic acidemia. Mol Genet Metab 2004;81(4):335–342. DOI: 10.1016/j.ymgme.2004.01.003.
98. Lucke T, Perez-Cerda C, Baumgartner M, et al. Propionic acidemia: Unusual course with late onset and fatal outcome. Metabolism 2004;53(6):809–810. DOI: 10.1016/j.metabol.2003.12.025.
99. van Karnebeek CD, Sly WS, Ross CJ, et al. Mitochondrial carbonic anhydrase VA deficiency resulting from CA5A alterations presents with hyperammonemia in early childhood. Am J Hum Genet 2014;94(3):453–461. DOI: 10.1016/j.ajhg.2014.01.006.
100. Canda E, Ucar SK, Coker M. Biotinidase deficiency: Prevalence, impact and management strategies. Pediatric Health Med Ther 2020;11:127–133. DOI: 10.2147/PHMT.S198656.
101. Stanescu S, Belanger-Quintana A, Fernandez-Felix BM, et al. Severe anemia in patients with propionic acidemia is associated with branched-chain amino acid imbalance. Orphanet J Rare Dis 2021;16(1):226. DOI: 10.1186/s13023-021-01865-7.
102. Alexopoulos SP, Matsuoka L, Hafberg E, et al. Liver transplantation for propionic acidemia: A multicenter-linked database analysis. J Pediatr Gastroenterol Nutr 2020;70(2):178–182. DOI: 10.1097/MPG.0000000000002534.
103. Grunert SC, Mullerleile S, De Silva L, et al. Propionic acidemia: Clinical course and outcome in 55 pediatric and adolescent patients. Orphanet J Rare Dis 2013;8:6. DOI: 10.1186/1750-1172-8-6.
104. Mutze U, Henze L, Gleich F, et al. Newborn screening and disease variants predict neurological outcome in isovaleric aciduria. J Inherit Metab Dis 2021;44(4):857–870. DOI: 10.1002/jimd.12364.
105. Mutze U, Henze L, Schroter J, et al. Isovaleric aciduria identified by newborn screening: Strategies to predict disease severity and stratify treatment. J Inherit Metab Dis 2023. DOI: 10.1002/jimd.12653.
106. Zhao Y, Zhu S, Huang X. [Current understanding and progress of research on isovaleric acidemia]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2022;39(1):99–102. DOI: 10.3760/cma.j.cn511374-20200616-00443.
107. Ensenauer R, Vockley J, Willard JM, et al. A common mutation is associated with a mild, potentially asymptomatic phenotype in patients with isovaleric acidemia diagnosed by newborn screening. Am J Hum Genet 2004;75(6):1136–1142. DOI: 10.1086/426318.
108. Schlune A, Riederer A, Mayatepek E, et al. Aspects of Newborn Screening in Isovaleric Acidemia. Int J Neonatal Screen 2018;4(1):7. DOI: 10.3390/ijns4010007.
109. Dubiel B, Dabrowski C, Wetts R, et al. Complementation studies of isovaleric acidemia and glutaric aciduria type II using cultured skin fibroblasts. J Clin Invest 1983;72(5):1543–1552. DOI: 10.1172/JCI111113.
110. Chinen Y, Nakamura S, Tamashiro K, et al. Isovaleric acidemia: Therapeutic response to supplementation with glycine, l-carnitine, or both in combination and a 10-year follow-up case study. Mol Genet Metab Rep 2017;11:2–5. DOI: 10.1016/j.ymgmr.2017.03.002.
111. Nizon M, Ottolenghi C, Valayannopoulos V, et al. Long-term neurological outcome of a cohort of 80 patients with classical organic acidurias. Orphanet J Rare Dis 2013;8:148. DOI: 10.1186/1750-1172-8-148.
112. Arnold GL, Salazar D, Neidich JA, et al. Outcome of infants diagnosed with 3-methyl-crotonyl-CoA-carboxylase deficiency by newborn screening. Mol Genet Metab 2012;106(4):439-41. DOI: 10.1016/j.ymgme.2012.04.006.
113. Forsyth R, Vockley CW, Edick MJ, et al. Outcomes of cases with 3-methylcrotonyl-CoA carboxylase (3-MCC) deficiency - Report from the Inborn Errors of Metabolism Information System. Mol Genet Metab 2016;118(1):15-20. DOI: 10.1016/j.ymgme.2016.02.002.
114. Grunert SC, Stucki M, Morscher RJ, et al. 3-methylcrotonyl-CoA carboxylase deficiency: Clinical, biochemical, enzymatic and molecular studies in 88 individuals. Orphanet J Rare Dis 2012;7:31. DOI: 10.1186/1750-1172-7-31.
115. Suormala T, Fowler B, Duran M, et al. Five patients with a biotin-responsive defect in holocarboxylase formation: Evaluation of responsiveness to biotin therapy in vivo and comparative biochemical studies in vitro. Pediatr Res 1997;41(5):666–673. DOI: 10.1203/00006450-199705000-00011.
116. Ingaramo M, Beckett D. Distinct amino termini of two human HCS isoforms influence biotin acceptor substrate recognition. J Biol Chem 2009;284(45):30862-70. DOI: 10.1074/jbc.M109.046201.
117. Cozzolino C, Villani GR, Frisso G, et al. Biochemical and molecular characterization of 3-Methylcrotonylglycinuria in an Italian asymptomatic girl. Genet Mol Biol 2018;41(2):379–385. DOI: 10.1590/1678-4685-GMB-2017-0093.
118. Friebel D, von der Hagen M, Baumgartner ER, et al. The first case of 3-methylcrotonyl-CoA carboxylase (MCC) deficiency responsive to biotin. Neuropediatrics 2006;37(2):72–78. DOI: 10.1055/s-2006-924024.
119. Thomsen JA, Lund AM, Olesen JH, et al. Is L-carnitine supplementation beneficial in 3-methylcrotonyl-CoA carboxylase deficiency? JIMD Rep 2015;21:79–88. DOI: 10.1007/8904_2014_393.
120. Gunay-Aygun M. 3-Methylglutaconic aciduria: a common biochemical marker in various syndromes with diverse clinical features. Mol Genet Metab 2005;84(1):1–3. DOI: 10.1016/j.ymgme.2004.12.003.
121. Jones DE, Jennings EA, Ryan RO. Diversion of Acetyl CoA to 3-Methylglutaconic Acid Caused by Discrete Inborn Errors of Metabolism. Metabolites 2022;12(5). DOI: 10.3390/metabo12050377.
122. Jones DE, Klacking E, Ryan RO. Inborn errors of metabolism associated with 3-methylglutaconic aciduria. Clin Chim Acta 2021;522:96–104. DOI: 10.1016/j.cca.2021.08.016.
123. Spergel CD, Milko M, Edwards C, et al. 3-Methylglutaconyl-Coenzyme-A Hydratase Deficiency and the Development of Dilated

Cardiomyopathy. Cardiol Res 2014;5(5):158–162. DOI: 10.14740/cr359w.
124. Hertzog A, Selvanathan A, Pandithan D, et al. 3-Methylglutaconyl-CoA hydratase deficiency: When ascertainment bias confounds a biochemical diagnosis. JIMD Rep 2022;63(6):568–574. DOI: 10.1002/jmd2.12332.
125. Clarke SL, Bowron A, Gonzalez IL, et al. Barth syndrome. Orphanet J Rare Dis 2013;8:23. DOI: 10.1186/1750-1172-8-23.
126. Chin MT, Conway SJ. Role of Tafazzin in Mitochondrial Function, Development and Disease. J Dev Biol 2020;8(2). DOI: 10.3390/jdb8020010.
127. Schlame M. Cardiolipin remodeling and the function of tafazzin. Biochim Biophys Acta 2013;1831(3):582–588. DOI: 10.1016/j.bbalip.2012.11.007.
128. Finsterer J. Barth syndrome: Mechanisms and management. Appl Clin Genet 2019;12:95–106. DOI: 10.2147/TACG.S171481.
129. Maheshwari A. Neutropenia in the newborn. Curr Opin Hematol 2014;21(1):43–49. DOI: 10.1097/MOH.0000000000000010.
130. Ferri L, Donati MA, Funghini S, et al. New clinical and molecular insights on Barth syndrome. Orphanet J Rare Dis 2013;8:27. DOI: 10.1186/1750-1172-8-27.
131. Steward CG, Groves SJ, Taylor CT, et al. Neutropenia in Barth syndrome: Characteristics, risks, and management. Curr Opin Hematol 2019;26(1):6–15. DOI: 10.1097/MOH.0000000000000472.
132. Yahalom G, Anikster Y, Huna-Baron R, et al. Costeff syndrome: Clinical features and natural history. J Neurol 2014;261(12):2275–2282. DOI: 10.1007/s00415-014-7481-x.
133. Ho G, Walter JH, Christodoulou J. Costeff optic atrophy syndrome: New clinical case and novel molecular findings. J Inherit Metab Dis 2008;31 Suppl 2:S419–S423. DOI: 10.1007/s10545-008-0981-z.
134. Anikster Y, Kleta R, Shaag A, et al. Type III 3-methylglutaconic aciduria (optic atrophy plus syndrome, or Costeff optic atrophy syndrome): Identification of the OPA3 gene and its founder mutation in Iraqi Jews. Am J Hum Genet 2001;69(6):1218–1224. DOI: 10.1086/324651.
135. Wortmann SB, Rodenburg RJ, Jonckheere A, et al. Biochemical and genetic analysis of 3-methylglutaconic aciduria type IV: A diagnostic strategy. Brain 2009;132(Pt 1):136–146. DOI: 10.1093/brain/awn296.
136. Al Tuwaijri A, Alyafee Y, Alharbi M, et al. Novel homozygous pathogenic mitochondrial DNAJC19 variant in a patient with dilated cardiomyopathy and global developmental delay. Mol Genet Genomic Med 2022;10(8):e1969. DOI: 10.1002/mgg3.1969.
137. Richter-Dennerlein R, Korwitz A, Haag M, et al. DNAJC19, a mitochondrial cochaperone associated with cardiomyopathy, forms a complex with prohibitins to regulate cardiolipin remodeling. Cell Metab 2014;20(1):158–171. DOI: 10.1016/j.cmet.2014.04.016.
138. Morgan K, Holmes TM, Schlaut J, et al. Genetic variability of HLA in the Dariusleut Hutterites. A comparative genetic analysis of the Hutterities, the Amish, and other selected Caucasian populations. Am J Hum Genet 1980;32(2):246–257. PMID: 7386460.
139. Baumgartner ER, Suormala T. Multiple carboxylase deficiency: Inherited and acquired disorders of biotin metabolism. Int J Vitam Nutr Res 1997;67(5):377–384. PMID: 9350481.
140. Tammachote R, Janklat S, Tongkobpetch S, et al. Holocarboxylase synthetase deficiency: novel clinical and molecular findings. Clin Genet 2010;78(1):88–93. DOI: 10.1111/j.1399-0004.2009.01357.x.
141. Zempleni J, Liu D, Camara DT, et al. Novel roles of holocarboxylase synthetase in gene regulation and intermediary metabolism. Nutr Rev 2014;72(6):369–376. DOI: 10.1111/nure.12103.
142. Tong L. Structure and function of biotin-dependent carboxylases. Cell Mol Life Sci 2013;70(5):863–891. DOI: 10.1007/s00018-012-1096-0.
143. Pestinger V, Wijeratne SS, Rodriguez-Melendez R, et al. Novel histone biotinylation marks are enriched in repeat regions and participate in repression of transcriptionally competent genes. J Nutr Biochem 2011;22(4):328–333. DOI: 10.1016/j.jnutbio.2010.02.011.
144. Solorzano-Vargas RS, Pacheco-Alvarez D, Leon-Del-Rio A. Holocarboxylase synthetase is an obligate participant in biotin-mediated regulation of its own expression and of biotin-dependent carboxylases mRNA levels in human cells. Proc Natl Acad Sci USA 2002;99(8):5325–5330. DOI: 10.1073/pnas.082097699.
145. Xia M, Malkaram SA, Zempleni J. Three promoters regulate the transcriptional activity of the human holocarboxylase synthetase gene. J Nutr Biochem 2013;24(11):1963–1969. DOI: 10.1016/j.jnutbio.2013.06.007.
146. Bao B, Pestinger V, Hassan YI, et al. Holocarboxylase synthetase is a chromatin protein and interacts directly with histone H3 to mediate biotinylation of K9 and K18. J Nutr Biochem 2011;22(5):470–475. DOI: 10.1016/j.jnutbio.2010.04.001.
147. Li Y, Hassan YI, Moriyama H, et al. Holocarboxylase synthetase interacts physically with euchromatic histone-lysine N-methyltransferase, linking histone biotinylation with methylation events. J Nutr Biochem 2013;24(8):1446–1452. DOI: 10.1016/j.jnutbio.2012.12.003.
148. Zempleni J, Hassan YI, Wijeratne SS. Biotin and biotinidase deficiency. Expert Rev Endocrinol Metab 2008;3(6):715–724. DOI: 10.1586/17446651.3.6.715.
149. Hymes J, Fleischhauer K, Wolf B. Biotinylation of histones by human serum biotinidase: assessment of biotinyl-transferase activity in sera from normal individuals and children with biotinidase deficiency. Biochem Mol Med 1995;56(1):76–83. DOI: 10.1006/bmme.1995.1059.
150. Hart PS, Hymes J, Wolf B. Isoforms of human serum biotinidase. Clin Chim Acta 1991;197(3):257–264. DOI: 10.1016/0009-8981(91)90146-4.
151. Wolf B. Clinical issues and frequent questions about biotinidase deficiency. Mol Genet Metab 2010;100(1):6–13. DOI: 10.1016/j.ymgme.2010.01.003.
152. Cowan TM, Kazerouni NN, Dharajiya N, et al. Increased incidence of profound biotinidase deficiency among Hispanic newborns in California. Mol Genet Metab 2012;106(4):485–487. DOI: 10.1016/j.ymgme.2012.05.017.
153. Wolf B. Biotinidase Deficiency. 2023. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle. Available from: https://www.ncbi.nlm.nih.gov/books/NBK1322/.
154. Hymes J, Stanley CM, Wolf B. Mutations in BTD causing biotinidase deficiency. Hum Mutat 2001;18(5):375–381. DOI: 10.1002/humu.1208.
155. Pindolia K, Jordan M, Wolf B. Analysis of mutations causing biotinidase deficiency. Hum Mutat 2010;31(9):983–991. DOI: 10.1002/humu.21303.
156. Sivri HS, Genc GA, Tokatli A, et al. Hearing loss in biotinidase deficiency: genotype-phenotype correlation. J Pediatr 2007;150(4):439–442. DOI: 10.1016/j.jpeds.2007.01.036.
157. Jezela-Stanek A, Suchon L, Sobczynska-Tomaszewska A, et al. Molecular Background and Disease Prevalence of Biotinidase Deficiency in a Polish Population-Data Based on the National Newborn Screening Programme. Genes (Basel) 2022;13(5). DOI: 10.3390/genes13050802.
158. Strovel ET, Cowan TM, Scott AI, et al. Laboratory diagnosis of biotinidase deficiency, 2017 update: A technical standard and guideline of the American College of Medical Genetics and Genomics. Genet Med 2017;19(10). DOI: 10.1038/gim.2017.84.
159. Bhardwaj P, Kaushal RK, Chandel A. Biotinidase deficiency: A treatable cause of infantile seizures. J Pediatr Neurosci 2010;5(1):82–83. DOI: 10.4103/1817-1745.66660.
160. Saleem H, Simpson B. Biotinidase Deficiency. 2023. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing.
161. Pomponio RJ, Hymes J, Pandya A, et al. Prenatal diagnosis of heterozygosity for biotinidase deficiency by enzymatic and molecular analyses. Prenat Diagn 1998;18(2):117–122. PMID: 9516011.
162. Lin Y, Wang W, Lin C, et al. Biochemical and molecular features of Chinese patients with glutaric acidemia type 1 detected through newborn screening. Orphanet J Rare Dis 2021;16(1):339. DOI: 10.1186/s13023-021-01964-5.
163. Strauss KA, Williams KB, Carson VJ, et al. Glutaric acidemia type 1: Treatment and outcome of 168 patients over three decades. Mol Genet Metab 2020;131(3):325–340. DOI: 10.1016/j.ymgme.2020.09.007.
164. Vester ME, Bilo RA, Karst WA, et al. Subdural hematomas: Glutaric aciduria type 1 or abusive head trauma? A systematic review. Forensic Sci Med Pathol 2015;11(3):405–415. DOI: 10.1007/s12024-015-9698-0.

165. Larson A, Goodman S. Glutaric Acidemia Type 1. 2022. In: GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle.
166. Sen A, Pillay RS. Striatal necrosis in type 1 glutaric aciduria: Different stages in two siblings. J Pediatr Neurosci 2011;6(2):146–148. DOI: 10.4103/1817-1745.92845.
167. Pinto PL, Camara B, Florindo C, et al. Glutaric Acidemia Type 1: Diagnosis, Clinical features, and Outcome in a Portuguese Cohort. Endocr Metab Immune Disord Drug Targets 2023. DOI: 10.2174/1871530323666230914122946.
168. Boy N, Muhlhausen C, Maier EM, et al. Recommendations for diagnosing and managing individuals with glutaric aciduria type 1: Third revision. J Inherit Metab Dis 2023;46(3):482–519. DOI: 10.1002/jimd.12566.
169. Li Q, Yang C, Feng L, et al. Glutaric Acidemia, Pathogenesis and Nutritional Therapy. Front Nutr 2021;8:704984. DOI: 10.3389/fnut.2021.704984.
170. Bjugstad KB, Goodman SI, Freed CR. Age at symptom onset predicts severity of motor impairment and clinical outcome of glutaric acidemia type 1. J Pediatr 2000;137(5):681–686. DOI: 10.1067/mpd.2000.108954.
171. Healy L, O'Shea M, McNulty J, et al. Glutaric aciduria type 1: Diagnosis, clinical features and long-term outcome in a large cohort of 34 Irish patients. JIMD Rep 2022;63(4):379–387. DOI: 10.1002/jmd2.12302.
172. Sharawat IK, Dawman L. Glutaric aciduria type 1 with microcephaly: Masquerading as spastic cerebral palsy. J Pediatr Neurosci 2018;13(3):349–351. DOI: 10.4103/JPN.JPN_79_17.
173. Rai SP. Glutaric aciduria type1: CT diagnosis. J Pediatr Neurosci 2009;4(2):143. DOI: 10.4103/1817-1745.57338.
174. Accogli A, Geraldo AF, Piccolo G, et al. Diagnostic approach to macrocephaly in children. Front Pediatr 2021;9:794069. DOI: 10.3389/fped.2021.794069.
175. Hoshino H, Kubota M. Canavan disease: Clinical features and recent advances in research. Pediatr Int 2014;56(4):477–483. DOI: 10.1111/ped.12422.
176. Kaul R, Gao GP, Aloya M, et al. Canavan disease: mutations among Jewish and non-Jewish patients. Am J Hum Genet 1994;55(1):34–41. PMID: 8023850.
177. Moffett JR, Ross B, Arun P, et al. N-Acetylaspartate in the CNS: from neurodiagnostics to neurobiology. Prog Neurobiol 2007;81(2):89–131. DOI: 10.1016/j.pneurobio.2006.12.003.
178. Ahmed SS, Gao G. Making the white matter matters: progress in understanding Canavan's disease and therapeutic interventions through eight decades. JIMD Rep 2015;19:11–22. DOI: 10.1007/8904_2014_356.
179. Appu AP, Moffett JR, Arun P, et al. Increasing N-acetylaspartate in the Brain during Postnatal Myelination Does Not Cause the CNS Pathologies of Canavan Disease. Front Mol Neurosci 2017;10:161. DOI: 10.3389/fnmol.2017.00161.
180. Rossler L, Lemburg S, Weitkamper A, et al. Canavan's spongiform leukodystrophy (Aspartoacylase deficiency) with emphasis on sonographic features in infancy: Description of a case report and review of the literature. J Ultrasound 2023;26(4):757–764. DOI: 10.1007/s40477-022-00667-2.
181. Bley A, Denecke J, Kohlschutter A, et al. The natural history of Canavan disease: 23 new cases and comparison with patients from literature. Orphanet J Rare Dis 2021;16(1):227. DOI: 10.1186/s13023-020-01659-3.
182. Corti M, Byrne BJ, Gessler DJ, et al. Adeno-associated virus-mediated gene therapy in a patient with Canavan disease using dual routes of administration and immune modulation. Mol Ther Methods Clin Dev 2023;30:303–314. DOI: 10.1016/j.omtm.2023.06.001.
183. Ahmed S, Siddiqui A, DeBerardinis RJ, et al. L-2-hydroxyglutaric aciduria - review of literature and case series. Ann Med Surg (Lond) 2023;85(4):712–717. DOI: 10.1097/MS9.0000000000000326.
184. Kranendijk M, Struys EA, Salomons GS, et al. Progress in understanding 2-hydroxyglutaric acidurias. J Inherit Metab Dis 2012;35(4):571–587. DOI: 10.1007/s10545-012-9462-5.
185. Seijo-Martinez M, Navarro C, Castro del Rio M, et al. L-2-hydroxyglutaric aciduria: Clinical, neuroimaging, and neuropathological findings. Arch Neurol 2005;62(4):666–670. DOI: 10.1001/archneur.62.4.666.
186. Ye D, Guan KL, Xiong Y. Metabolism, Activity, and Targeting of D- and L-2-Hydroxyglutarates. Trends Cancer 2018;4(2):151–165. DOI: 10.1016/j.trecan.2017.12.005.
187. Ullah MI, Nasir A, Ahmad A, et al. Identification of novel L2HGDH mutation in a large consanguineous Pakistani family-A case report. BMC Med Genet 2018;19(1):25. DOI: 10.1186/s12881-018-0532-x.
188. Srinivasaraghavan R, Sharma S, Kratz L, et al. Child with D-2-hydroxyglutaric aciduria type II: A rare neurometabolic disorder. Ann Indian Acad Neurol 2021;24(6):933–934. DOI: 10.4103/aian.AIAN_231_20.
189. Park KC, Krywawych S, Richard E, et al. Cardiac Complications of propionic and other inherited organic acidemias. Front Cardiovasc Med 2020;7:617451. DOI: 10.3389/fcvm.2020.617451.

REVIEW ARTICLE

Transcranial Doppler: A New Stethoscope–Voiceover Tool for Neonatal Brain

Gunjana Kumar[1], Jyoti Patodia[2], Nirmal Chandra Padhan[3], Akhil Maheshwari[4,5], Pradeep Suryawanshi[6]

Received on: 20 October 2023; *Accepted on*: 20 November 2023; *Published on*: 05 January 2024

Abstract

Background: Cerebral Doppler ultrasound is an emerging bedside tool to measure cerebral perfusion in premature and critically ill neonates. The review focused on maturation and disease-associated Doppler spectra in neonates for diagnostic and prognostic relevance and to identify relevant research areas.

Methods: A comprehensive literature search was conducted to review cerebral Doppler parameters noted in specific disease states. Further efforts were focused to understand the clinical relevance of these indices in the management of neonates and to predict their long-term neurodevelopmental outcomes.

Results: The review focused on routinely used cerebral Doppler parameters in normal and diseased states. Resistive index (RI) in the anterior cerebral artery (ACA) is a frequently used parameter in infants with primary brain injury and in preterm neonates with hemodynamically significant patent ductus arteriosus (HsPDA).

Conclusion: Despite extensive use, major gaps remain in our understanding of cerebral Doppler parameters for diagnosis, monitoring, and prediction of neurodevelopmental outcomes in neonates. Further studies are needed to decode these data in a more precise manner.

Keywords: Acoustic windows, Anterior cerebral artery, Anterior fontanelle, Area under the velocity curve, Basilar artery, Brain injury, Continuous-wave Doppler, Convex probe, Cerebral autoregulation, Cerebral blood flow, Cerebral Doppler, Cerebral Doppler ultrasound, Doppler index, Doppler waveform, Hemodynamically significant patent ductus arteriosus, End-diastolic velocity, High-frequency linear transducer, Hypoxic–ischemic encephalopathy, Neuroanatomy, Intrauterine growth restriction, Intraventricular hemorrhage, Kangaroo mother care, Line of insonation, Liquefactive necrosis, Neonate, Peak-systolic velocity, Plial arterioles, Pulse-wave Doppler signal, Pulsatility index, Resistive index, Vein of Galen malformation.

Newborn (2023): 10.5005/jp-journals-11002-0077

Key Points

- Cerebral Doppler ultrasound is an emerging bedside tool for measuring cerebral perfusion in premature and critically ill neonates.
- Several parameters of cerebral Doppler are important in distinguishing normal from diseased states. Resistive index (RI) in the anterior cerebral artery (ACA) is frequently used to monitor primary brain injury in term and preterm neonates with hemodynamically significant patent *ductus arteriosus*.
- The article summarizes maturation- and disease-associated Doppler spectra in neonates for diagnostic and prognostic relevance and to identify relevant research areas.
- Further information is needed to determine the impact of cerebral Doppler indices on long-term neurodevelopmental outcomes.

Introduction

Cerebral Doppler ultrasound, a comprehensive clinical vital appliance of neonatal intensive care practice, has been utilized extensively to assess the vascular anatomy and hemodynamics of the brain in small and sick neonates. It has been used to diagnose and predict outcomes of neonatal diseases like intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), hemodynamically significant patent ductus arteriosus (HsPDA), hydrocephalus, sepsis, shock, hypoxic–ischemic encephalopathy (HIE), and vascular malformations. Consequently, Doppler imaging has gained importance in day-to-day clinical practice, especially in infants with impaired cerebral autoregulation.[1] A basic understanding of neuroanatomy through acoustic windows of fontanelles using appropriate Doppler techniques in cerebral

[1]Department of Neonatology, National Institute of Medical Sciences, Jaipur, Rajasthan, India
[2]Department of Neonatology, Hope Children Hospital, Jaipur, Rajasthan, India
[3]Department of Neonatology, Shishu Bhawan Hospital, Bilaspur, Chattisgarh, India
[4]Department of Pediatrics, Louisville State University, Shreveport, Louisville, United States of America
[5]Global Newborn Society, Clarksville, Maryland, United States of America
[6]Department of Neonatology, Bharati Vidyapeeth University Medical College, Pune, Maharashtra, India
Corresponding Author: Pradeep Suryawanshi, Department of Neonatology, Bharati Vidyapeeth University Medical College Hospital and Research Center, Pune, Maharashtra, India, Phone: +91 9923540500, e-mail: drpradeepsuryawanshi@gmail.com

How to cite this article: Kumar G, Patodia J, Padhan NC, *et al*. Transcranial Doppler: A New Stethoscope—Voiceover Tool for Neonatal Brain. Newborn 2023;2(4):279–290.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as 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 the Editor-in-Chief and his research group.

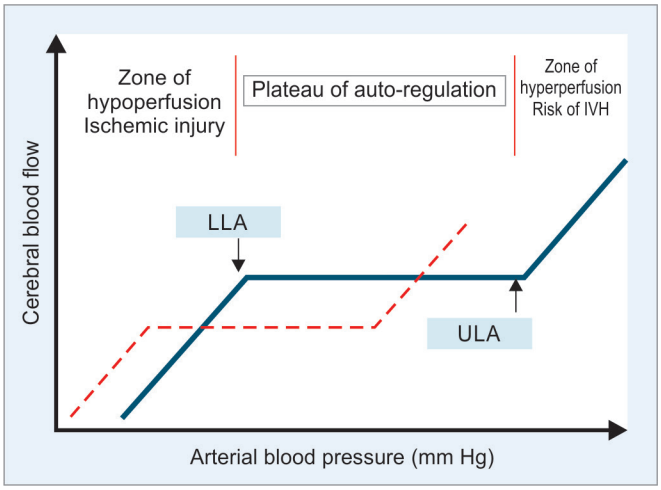


Fig. 1: Cerebral autoregulation sigmoid curve in term (blue) and preterm neonates (red): The flat area of the sigmoid curve is the autoregulatory plateau where cerebral perfusion pressure (CPP) is maintained by cerebral vasoreactivity. Beyond the upper limit of autoregulation (ULA) and the lower limit of autoregulation (LLA), cerebral blood flow (CBF) is passive to blood pressure due to impaired vasoreactivity. This infact is worse in preterm infants with a narrow plateau depicting poor autoregulation (red dotted line) (x-axis: mean arterial blood pressure, y-axis: CBF)

arteries is a reliable method to predict cerebral injury; real-time snapshot assessments can enable longitudinal accessibility and ensure response to treatment and follow-up.

Four decades ago, Bada et al.[2] outlined the role of cerebral Doppler imaging in diagnosing neonatal brain injury. Since then, various studies have evaluated the role of Doppler parameters and their correlation with the risk and severity of brain injury. However, we still need a systematic approach to apply Doppler evaluation of cerebral vascular anatomy and hemodynamics in clinical practice. In this article, we reviewed the available literature to identify these gaps for further study.

Physiology of Cerebral Blood Flow (CBF) Autoregulation and its Effect on Cerebral Doppler

Changes in CBF play a vital role in causing perinatal brain injury. Disturbances in cerebral circulation and autoregulation before, during, and after birth are believed to be major determinants of the severity of cerebral hemorrhage and HIE. Interestingly, systemic disorders such as shock, sepsis, and patent *ductus arteriosus*, which alter somatic hemodynamics, also affect cerebral circulation due to poorly defined autoregulatory mechanisms. Cerebral flow velocity is dependent on variables such as blood viscosity, cardiac output, pCO_2, pO_2, blood glucose levels, and the total oxygen content of arterial blood. Fluctuations in cerebral dynamics are driven mainly at the level of plial arterioles, and to a lesser extent, by large arteries.[3]

CBF = Cerebral perfusion pressure (CPP)/Cerebrovascular resistance (CVR) = [mean arterial pressure (MAP) – intracranial pressure (ICP)]/cerebrovascular resistance

The classic cerebral autoregulation is a sigmoid curve (Fig. 1), and the range of autoregulatory plateau is bound by the upper and lower limits of autoregulation (LA). Cerebral vasoreactivity functions only when mean arterial pressure (MAP) or cerebral perfusion pressure (CPP) is along this plateau. Above and below the range, CBF is passive to blood pressure, and the brain is at risk of hyperemic or hypoperfusion injury.[4] With the gradual development of this autoregulation from 26 to 33 weeks of gestation, the range of the autoregulation plateau is much narrower in preterm neonates (Fig. 1: red dotted line).

Table 1: Acoustic windows in cerebral Doppler that show various vessels and sinuses

Acoustic window	*Artery visualized*
Anterior fontanelle coronal plane	• Anterior cerebral artery (ACA) A1 segment (assessed most frequently) • Internal carotid artery (ICA) • Middle cerebral artery M1 segment (MCA) • Thalamostriate arteries • Cavernous sinus
Anterior fontanelle sagittal plane	• ACA; (assessed most frequently) • ICA • Basilar artery • Internal cerebral vein • Vein of Galen • Superior sagittal sinus • Sigmoid sinus
Temporal window	• MCA; (assessed most frequently) • Posterior cerebral artery

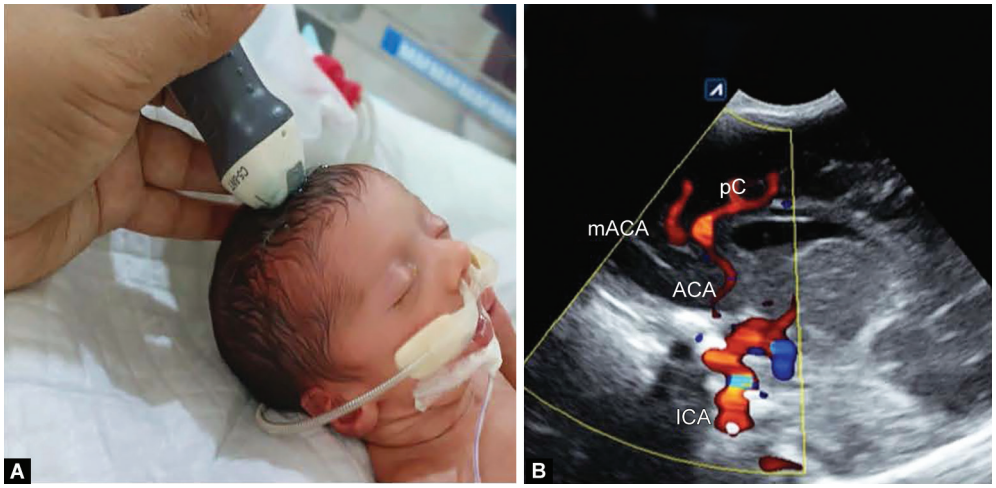
Technique and Parameters Used for Doppler Flow Measurements

To study the real-time blood flow patterns in the neonatal brain, we need a basic understanding of the altered anatomy and pathophysiology of the various disease conditions. Keeping the infant calm and comfortable while performing a Doppler flow study is important.

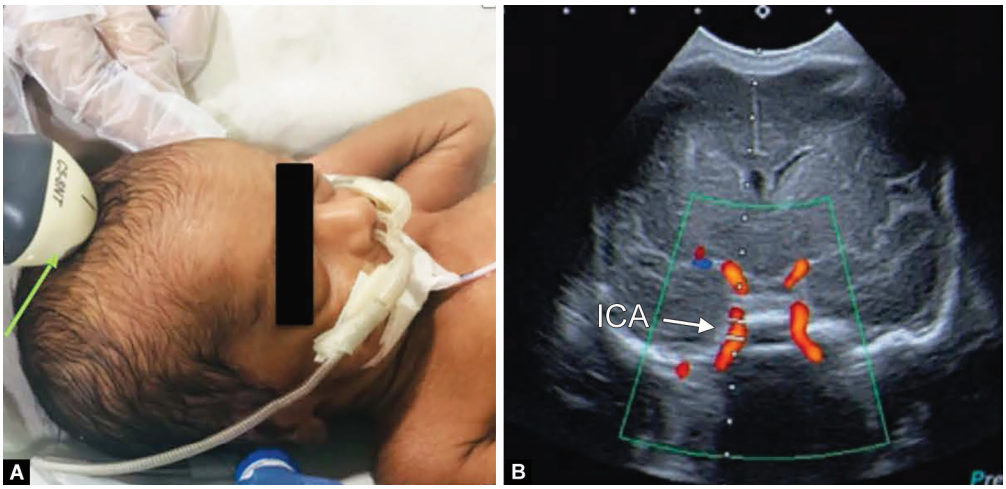
The professionals need to be familiar with ultrasound machines. Understanding the layout of these machines, particularly the components needed for color Doppler imaging (CDI), is important. Protocols are needed to correctly move the color box, obtain the pulse-wave (PW) Doppler signals, and correctly place the flow sample volumes on the artery to be investigated. In practice, we prefer high-frequency convex probes of 8–5 MHz. High-frequency linear transducers are useful to visualize superficial vessels at or near the brain's convexity, such as the superior sagittal and transverse sinuses. The acoustic windows for obtaining the images depend on the vessel and sinus of interest, anterior fontanelle, and temporal windows (Table 1).

Assessing CBF Pattern and Velocities

Doppler assesses the flow and not the velocity. One must always remember that blood flow in the cerebral artery should be in the forward direction: any reversal of flow is typically abnormal. Depending on the location, the color window superimposed on the gray scale depicts the blood flow in various cerebral vessels. The professionals should be familiar with the placement of the probe position according to the direction of blood flow in various cerebral vessels. Blood flowing toward the probe is seen as a red signal, whereas blood flowing away from the probe appears blue.



Figs 2A and B: Anterior fontanelle (AF) midsagittal view: (A) Position of the probe in the sagittal plane with the probe marker towards the nose; (B) Midline sagittal view showing the anterior cerebral artery (ACA) and its two branches, the pericallosal (pC) and marginal (mACA), along with the visualization of the internal carotid artery (ICA)



Figs 3A and B: Anterior fontanelle (AF) coronal view: (A) Note the position of the probe in the coronal plane with the probe marker towards the right (green arrow); (B) Coronal plane showing internal carotid artery (ICA)

There are some important points to remember:

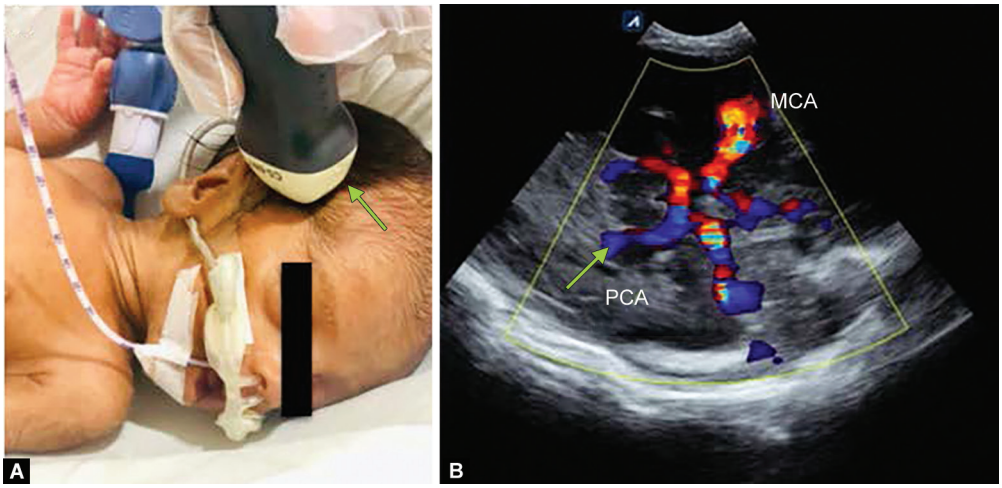
- Selection of the correct blood vessel for assessment;
- Placement of the color map over the area of interest. The measurements should be performed on a vessel running parallel to the line of insonation. This is because the angle between the axis of the blood vessel and the Doppler beam can affect the Doppler frequency shift and result in suboptimal quality of spectra. The angle of insonation must be kept close to zero by adjusting the angle of the probe;
- The most-frequently assessed blood vessels are the ACA and internal carotid artery via the anterior fontanelle (Figs 2 and 3), and the middle cerebral artery from the temporal window. The temporal window can also be used to visualize the posterior cerebral artery (Figs 4 and 5);
- The pulse-wave, not the continuous-wave Doppler, is better for studying vessels in the path of the ultrasound beam. Choosing continuous-wave Doppler can confuse the origin of the signal (Fig. 6);
- The Doppler waveform should be optimized to have a good-quality spectrum with fairly uniform signals;
- The peak-systolic and end-diastolic velocities are identified and the Doppler indices are measured. A mean of 3–5 cardiac cycles is usually taken for the measurement. Most ultrasound systems will automatically or semiautomatically generate the indices (Figs 5 and 6).
- The resistive index (RI) is the most frequently used Doppler index. It is defined as:

$$RI = \frac{\text{Peak-systolic velocity} - \text{end-diastolic velocity}}{\text{Peak-systolic velocity}}$$

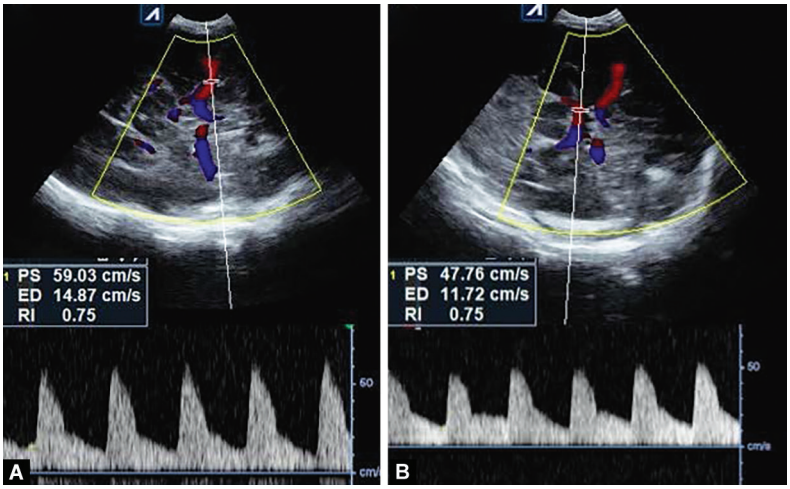
Serial measurements in the same vessel at the same location are useful in early identification of changes in the condition of the neonate. Some other flow parameters are described in Table 2.

Some Clinically Relevant Aspects of Cerebral Doppler

In the neonatal intensive care unit (NICU), factors such as gestational age, birth weight, postnatal day, small for gestational



Figs 4A and B: Temporal window: (A) Please note the position of the probe in the temporal area to visualize the middle cerebral artery (MCA) and posterior cerebral artery (PCA), probe marker (green arrow); (B) Temporal window showing a circle of Willis, MCA and PCA



Figs 5A and B: Temporal window showing Doppler study: (A) Doppler study of middle cerebral artery (MCA); red color of the flow shows its direction toward the probe. Pulse wave Doppler (PWD) showing peak systolic velocity (PSV), end-diastolic velocity (EDV), and resistive index (RI); (B) Temporal window showing posterior cerebral artery (PCA). PWD shows normal PSV, EDV, and RI

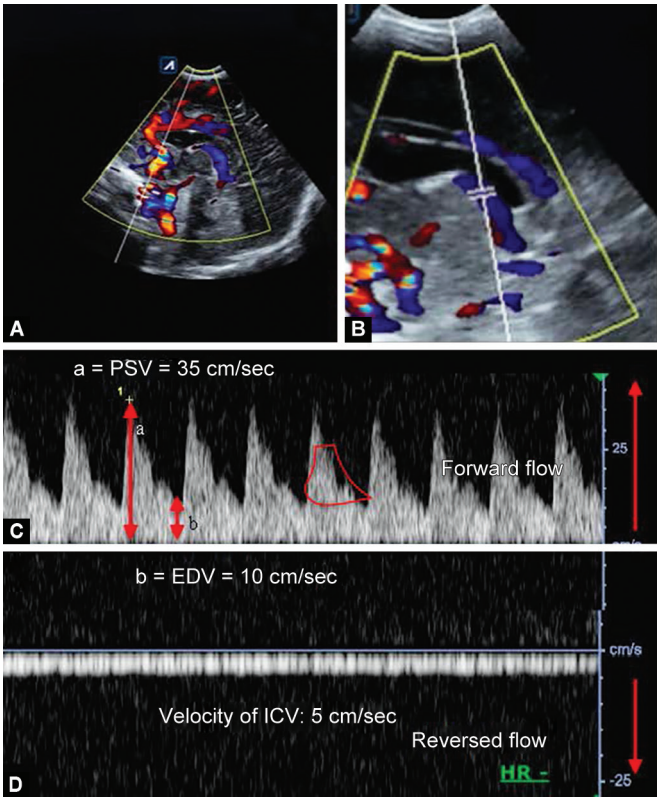
age,[5] pCO_2,[6] hematocrit,[7] sedation,[8] position of the baby,[9,10] and hypoglycemia[11] may influence the CBF and may lead to inconsistent results. Gestational age can affect CBF; some studies show a clear correlation with RI,[12] whereas others do not.[13] The RI in the ACA drops from a mean of 0.78 (range 0.65–0.85) in preterm infants to 0.7 (range 0.6–0.8) in the full-term neonates.[14–18] The increase in diastolic flow with postnatal age may result from decreasing cerebrovascular resistance or diminishing shunting as a result of ductal closure. Further, these data relate to the known downward trend of the RI during the first year after birth, especially after the closure of fontanelle when the mean RI decreases between 0.5 and 0.6.[18,19]

Impact of Gestational Age and Postnatal Days

Couture et al.[20] reported a progressive increase in peak-systolic velocity (PSV) and end-diastolic velocity (EDV) in the ACA, internal carotid artery (ICA), and the basilar artery from 32 weeks of gestation to 8 months of postnatal age, evolving both in parallel with gestational and postnatal age. In the clinical setting, relying only on RI could be misleading as a normal RI could be associated with low blood velocities (Fig. 7). Archer et al.[14] studied 24 full-term infants and reported a fall in the pulsatility index (PI) and increasing diastolic velocity in the first 5 days after birth.

Oxygen, CO_2, and Mean Arterial Pressure

With impaired cerebral autoregulation, the preterm brain is exposed to various stress responses, including those associated with changes in pO_2, pCO_2, and mean arterial blood pressure (MABP). Menke et al.[21] quantified the influence of these parameters in mechanically ventilated preterm infants (gestational age <33 weeks) on cerebral blood flow velocity (CBFV) measured in ICA, with a MABP reactivity of 7.5% (–12.5 to 20.1%), a rise in CBFV per 1 kPa rise in MABP was observed. Similarly, a pCO_2 reactivity of 32.7% (–8.1 to 79.5%) rise in CBFV/1 kPa rise in pCO_2, and a minimal pO_2 reactivity of –3.1% (–14.2 to 7.9%) fall in CBFV/1 kPa rise in pO_2 was reported. These findings show altered pCO_2 to be a major determinant of CBFV and hence, raise a possibility of its being one of the major causes of preterm brain injury.



Figs 6A to D: Doppler measurements: (A) Pulse wave Doppler (PWD) of the anterior cerebral artery (ACA) in midsagittal view seen via the anterior fontanelle (AF); (B) PWD in the internal cerebral vein (ICV) in midsagittal view (AF); (C) Forward flow positive deflection of the artery and its various measurements: a = PSV (peak systolic velocity, 35 cm/s) and b = EDV (end-diastolic velocity, 10 cm/s); (D) Reversed flow (negative deflection) in vein with velocity of 5 cm/s

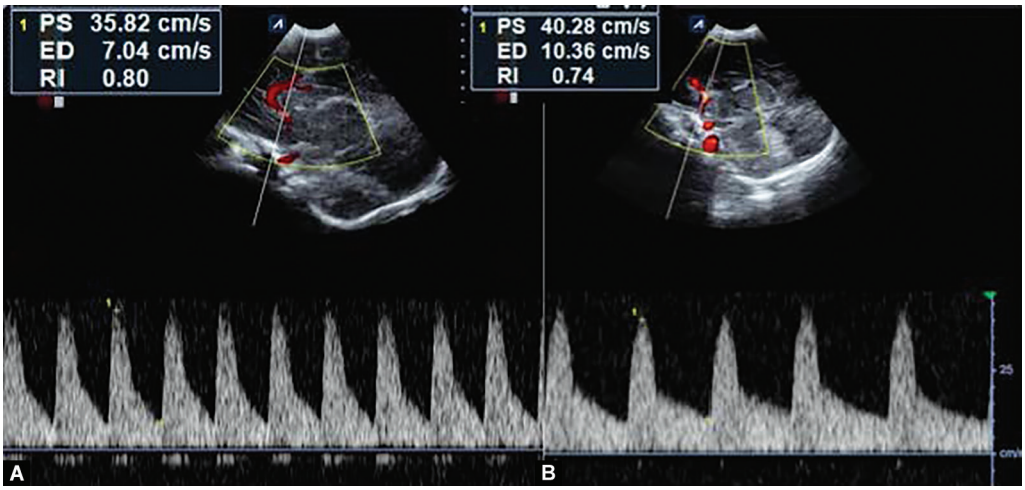
Cerebral Doppler in Various Pathological Conditions (Table 3)

Intraventricular/Intracerebral Hemorrhage

The absence of CBF autoregulation with hypoxic-reperfusion injury due to systemic hemodynamic instability has been implicated in the pathogenesis of IVH. A recent systematic review (2020, 5 studies) reported no significant correlation among Doppler parameters

Table 2: Doppler parameters

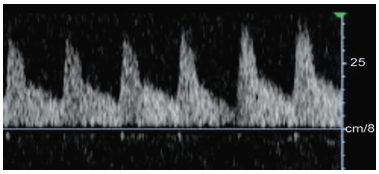
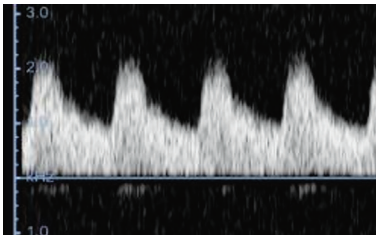
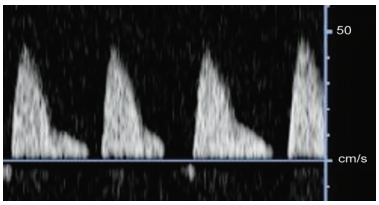
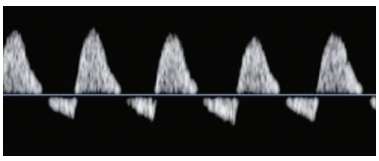
Doppler parameters	*Explanation*
Resistive index (RI)	Peak systolic velocity (PSV) – end diastolic velocity (EDV)/PSV
Pulsatility index (PI)	Peak systolic velocity – end diastolic velocity/mean velocity
Mean velocity (MV)	Mean velocity calculated over the series of cardiac cycles
Peak systolic velocity (PSV)	Highest velocity in the cardiac cycle
End diastolic velocity (EDV)	Lowest velocity in the cardiac cycle
Area under the velocity curve (AUVC)	Represents mean flow velocity
Cerebral blood flow fluctuation (CBFF)	Interquartile range of velocity
V_{mean} ratio	MV in first 12 h/MV at 12–168 h
Time averaged velocity (TAV)	$V_{max}/2$
Coefficient of variability (CV%)	Coefficient of variation of AUVC values of 20 consecutive cardiac cycles
Cerebral blood flow resistance	Cerebral perfusion pressure/RI



Figs 7A and B: Interpretation of flow velocities. (A) Case 1: seven-day-old term neonate with encephalopathy, PSV: 35 cm/s, EDW: 7 cm/s, and RI -0.8; (B) Case 2: Five-day-old 32 weeks preterm, PSV: 40 cm/s, EDV: 10 cm/s, and RI: 0.74. However, RI appears to be normal in both cases, measurement of velocities in case (A) represents low cerebral blood flow

EDV, end-diastolic velocity; PSV, real systolic velocity; RI, resistive index

Table 3: The pulse-wave Doppler of the anterior cerebral artery demonstrating variation in Doppler parameters, its clinical conditions and implications

Pulse-wave ACA Doppler	*Interpretation*	*Clinical conditions*	*Implication/Prognostication*
	RI: 0.74–0.85 Normal	Normal term/preterm	Abnormal RI <0.6 or >0.85
	RI: 0.6 Decreased A low RI: Lower vascular resistance and higher CBFV (increased diastolic flow) are a marker of a reperfusion injury.	• Early stage of asphyxia • Stroke • Vascular malformation • Neonatal seizures • Hypoxia • Hypercarbia • Hypotension	RI <0.6 in the initial 24 hours in case of birth asphyxia has 100% sensitivity and 86% specificity for adverse outcomes. Low RI in the first <72 hours of life could be associated with PVL or adverse neurological outcomes in preterm neonates. Serial monitoring of RI is more useful than a single reading.
	RI = 1 Increased A high RI indicates increased resistance and lower CBFV (decreased diastolic flow).	• HsPDA • IVH/ICH • Later stages of asphyxia (severe HIE) • PVL • Hydrocephalus • Sepsis/meningitis • Polycythemia	RI value >0.85 is seen in severe cases of asphyxia. Increased RI (>0.8) is associated with HsPDA or hydrocephalus.
	RI >1 Increased Reversed-diastolic flow: Loss of cerebral autoregulation.	• Late stage of severe asphyxia • Severe sepsis • Severe neonatal encephalopathy • Brain death	Reversed flow always a bad prognosticating marker.

HIE, hypoxic-ischemic encephalopathy; HsPDA, hemodynamically significant patent ductus arteriosus; RI, resistive index

(PSV, RI, PI, MV, or cerebral blood flow fluctuation) with preterm IVH or intracranial hemorrhage.[22] On the contrary, Van Bel et al. studied 60 preterm infants born prior to 34 weeks and noted the RI as being significantly lower and a reasonable correlation with the area under the velocity curve (AUVC). Infants who developed severe IVH had considerably higher variation in RI and AUVC in the first postnatal week.[23] A conclusive temporal relationship between Doppler changes and the onset of bleeding has not be established so far (Fig. 8).

Measurements of velocity changes in the perfusion waveform of the internal cerebral vein (ICV) have shown a promising association with IVH in ELBW babies.[24] The IVH rate is significantly higher in those with severe pulsatile flow or interrupted or reverse flow patterns in ICV than in others with normal, continuous flow (Fig. 9). Hence, changes in ICV Doppler may be a promising tool to predict severe IVH in preterm neonates and require further studies for its future implications.

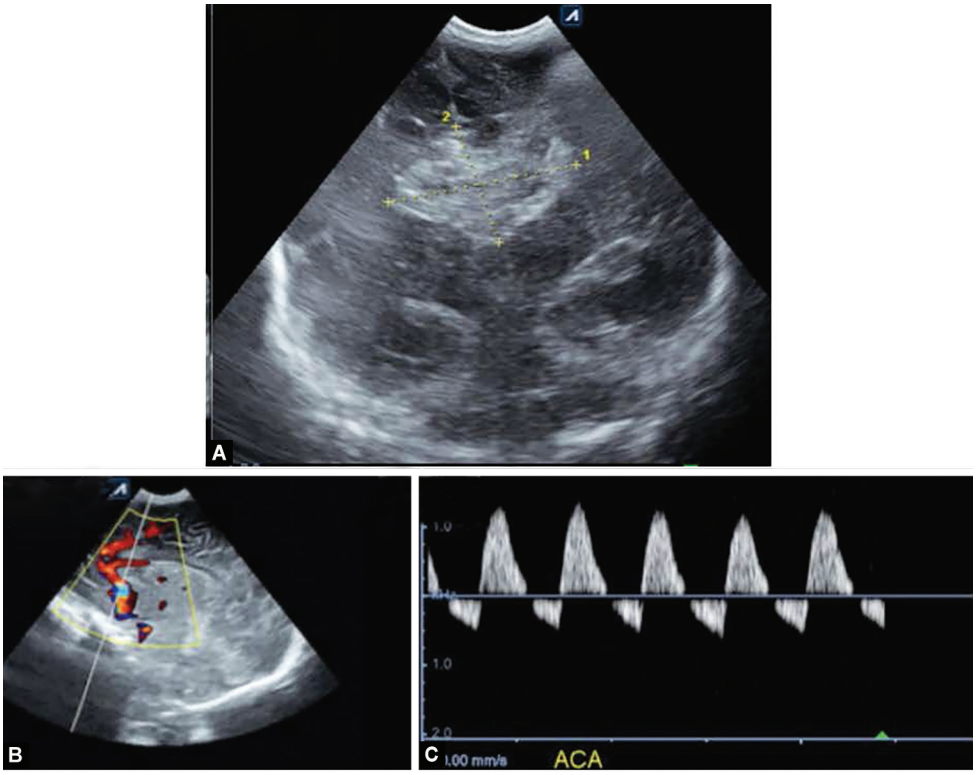
Periventricular Leukomalacia (PVL)

Okumura et al.[25] reported that ventilated preterm infants (27–34 weeks) who developed PVL had lower RI in the ACA during the first 72 hours after birth. In contrast, the CBFVs do not fall until beyond the 1st week after birth in the posterior cerebral artery and the ICA, and until beyond the 2nd week in the ACA, MCA, and basilar artery.[26] The total cerebral blood supply and the mean velocity fell soon after birth in all major cerebral arteries and continued to be low until 1–2 months in infants who went on to develop cystic PVL. A systematic review by Camfferman et al.[22] (5 studies) represented no correlation between CBFV and PVL (Fig. 10). We need further evaluation.

Perinatal Asphyxia

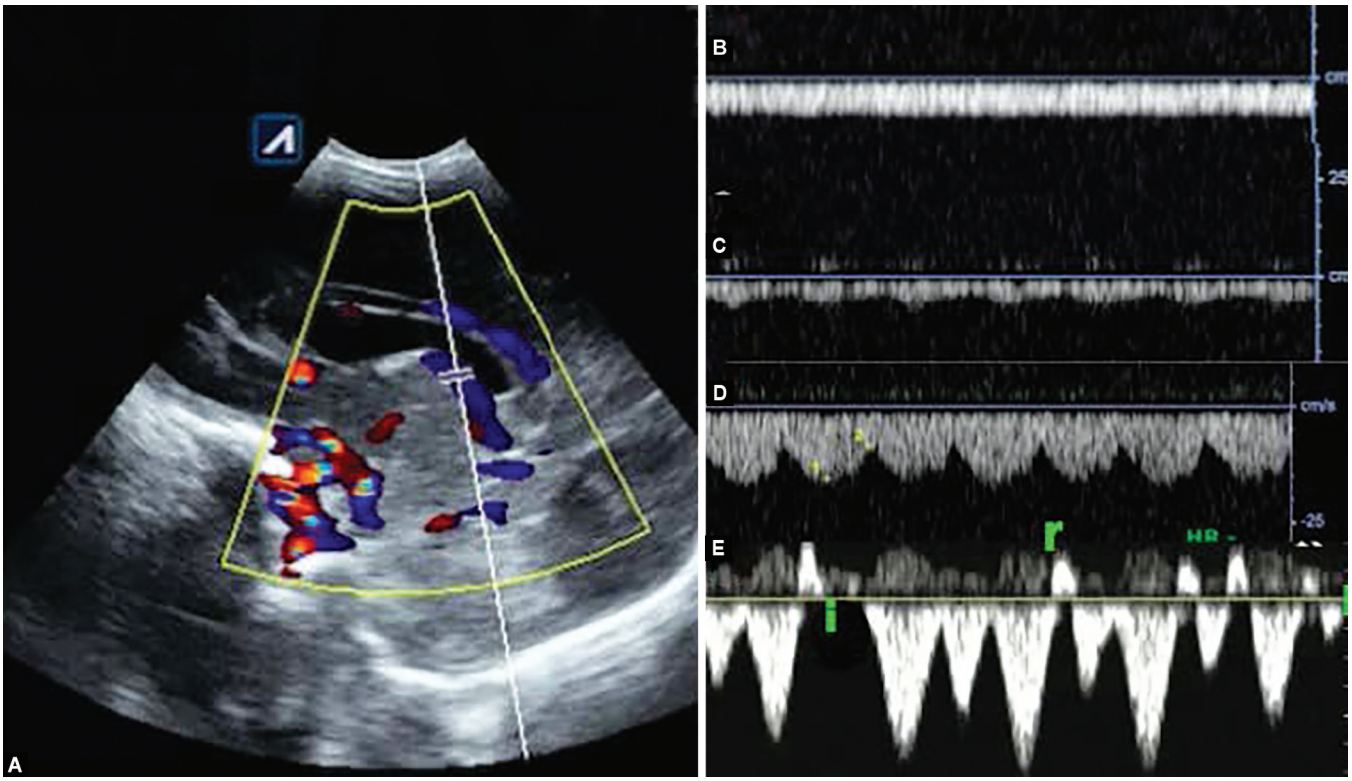
In perinatal asphyxia, there is an initial adaptive vasodilation to maintain oxygen delivery to the brain tissue. This leads to increased diastolic flow and a low RI (Fig. 11) indicating low vessel resistance and higher CBFV that is a marker of reperfusion injury. In severe asphyxia, the cerebral autoregulation may get impaired, leading to high RI (Fig. 11), and leads to increased vascular resistance. Natique et al.[27] noted an inverse correlation of RI in the MCA measured shortly after birth and the severity of encephalopathy. Neonates with mild HIE have been noted to show abnormal mean RIs, congruity-abnormal amplitude electroencephalography (45%), and abnormal brain magnetic resonance imaging (45%) and head ultrasound (44%).

Cerebral Doppler has been evaluated as a marker of adverse outcomes in cases of perinatal asphyxia now for several decades. In the late 1980s, Archer et al.[28] noted that abnormal Doppler (RI in ACA <0.55) can predict adverse outcomes (death or handicap) with

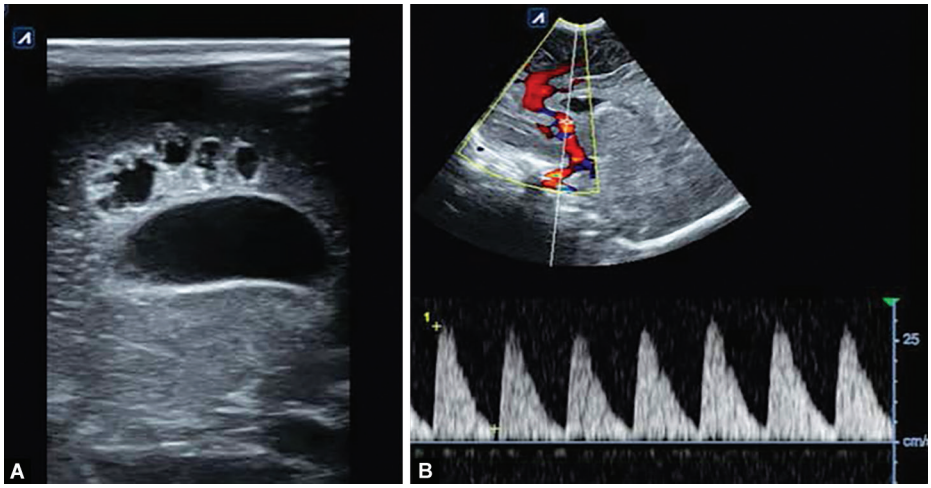


Figs 8A to C: Intracranial hemorrhage Doppler: (A) Term infant with large intraparenchymal hemorrhage measuring 3.5 × 2.3 cm in size; (B) PWD Doppler in ACA in midsagittal view; (C) Increased RI (reversed diastolic flow)

ACA, anterior cerebral artery; RI, resistive index; PWD, pulse wave Doppler

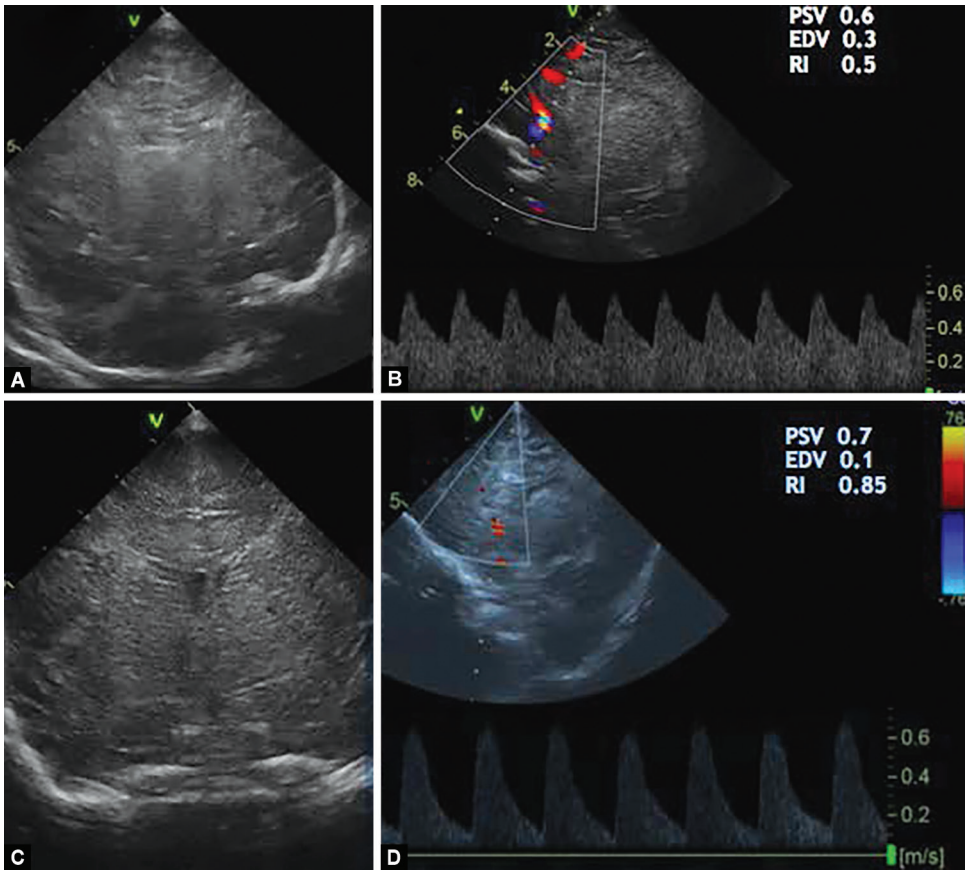


Figs 9A to E: (A) Pulse wave Doppler of the internal cerebral vein (blue flow) in sagittal view showing various venous waveform patterns; (B) Continuous flow pattern; (C) Mild pulsatile flow pattern; (D) Severe pulsatile flow pattern; (E) Show (i) interrupted or (r) reversed flow pattern. B and C patterns are normal, whereas the D and E patterns suggest decreased cerebral blood flow in neonates with a high risk of intraventricular hemorrhage



Figs 10A and B: Doppler study in a 1-month-old 27-week premature neonate with periventricular leukomalacia (PVL): (A) Parasagittal view: multiple cysts (3–4 mm) in frontal and parietal periventricular regions. The PVL was assessed as grade 3 with ventricular prominence; (B) Sagittal view PWD in ACA (red flow) showing increased RI: 0.9, decreased diastolic flow

ACA, anterior cerebral artery; RI, resistive index PWD; pulsed wave Doppler



Figs 11A to D: Term infant with a history of severe perinatal asphyxia. Ultrasound examination performed on day 1 showed: (A) Coronal view: prominent cerebral edema with obliterated ventricles; (B) Sagittal view PWD in ACA showing increased EDV and decreased RI (0.5) in early asphyxia; (C) Second case-term baby with asphyxia ultrasound examination performed on day 3, coronal view: prominent cerebral edema with obliterated ventricle; (D) Sagittal view PWD of ACA showing decreased EDV and increased RI (0.85)

ACA, anterior cerebral artery; EDV, end diastolic velocity; PWD, pulse wave Doppler; RI, resistive index

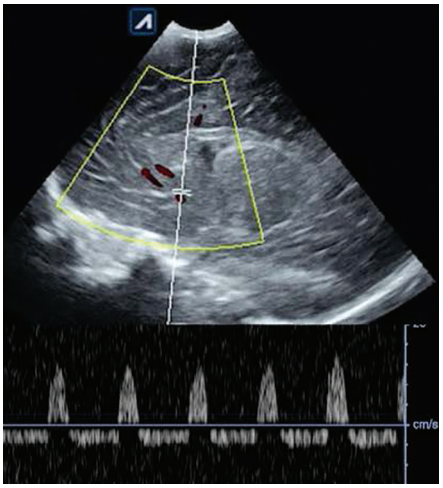


Fig. 12: Term infant with a history of severe perinatal asphyxia on postnatal day 4. PWD of ACA in sagittal view showed short systolic spikes and retrograde diastolic flow evolving to a brain death pattern

ACA, anterior cerebral artery; PSV, peak systolic velocity; PWD, pulsed wave Doppler

86% accuracy (100% sensitivity, 81% specificity). In another study, Liu et al.[29] classified the severity of asphyxiated brain injury based on cerebral Doppler parameters. Mild HIE was typically associated with an RI < 0.55 with significantly decreased blood flow velocity suggesting hypoperfusion. Some infants with moderate-to-severe HIE showed RI < 0.55 but had increased blood flow velocity (>2 SD), suggesting hyper-perfusion. Overall, low RIs were associated with severe HIE.

Elevated RI with decreased cerebral blood flow velocities, especially EDV, consistently indicates the diagnosis of HIE, with RI > 0.9 to be usually associated with severe encephalopathy. High RIs with the absence of blood flow during diastolic phases denote severe HIE. In cases with most severe HIE, there may be high RI with reversed diastolic flow. This inverse perfusion in cerebral tissues during diastolic phases indicates brain death (Fig. 12).

Rath et al.[30] systematically reviewed 26 studies and evaluated the importance of Doppler parameters in predicting long-term outcomes following perinatal asphyxia. They separately analyzed studies from the precooling and cooling eras. From the pretherapeutic era, pooled sensitivity and specificity, area under the receiver-operating characteristic curve, and diagnostic odds ratio of RI or CBFV for predicting "death or severe disability" were 0.83 [95% confidence interval (CI) 0.45–0.97] and 0.92 [95% CI 0.74–0.98], 0.94 [95% CI 0.92–0.96], and 54 [95% CI 7–391], respectively. Measurements from the therapeutic hypothermia era were 0.62 [95% CI 0.41–0.80] and 0.96 [95% CI 0.88–0.99], 0.93 [95% CI 0.89–0.94], and 23 [95% CI 6–91]. These measurements were taken before cooling was initiated. Studies measuring cerebral velocities during and after cooling represented the following outcomes, respectively: 0.51 [95% CI 0.24–0.78] and 0.83 [95% CI 0.73–0.90], 0.81 [95% CI 0.78–0.85], and 5 [95% CI 2–13]. Hence, cerebral Doppler may be useful in predicting death or disability in infants with HIE who are not cooled or if performed prior to cooling. However, the tests might not be valid if performed during or after cooling.

Sepsis

The data on the effects of sepsis on CBF show considerable variability. Some studies showed increased flow correlating to vasodilatation,[31,32] whereas others illustrated increased PI as representing decreased CBF.[33,34] These alterations in cerebral hemodynamics have been attributed to impaired blood–brain barrier secondary to the systemic inflammatory response via cytokines and endotoxins affecting cerebral circulation. Cerebral dysfunction may also be affected both due to cardiac dysfunction and because of microthrombi altering the autoregulatory mechanisms in the microcirculation. Increased CBF could well begin *in utero* prior to postnatal inflammatory changes. Hence, cerebral Doppler could serve as a surrogate diagnostic marker of early-onset sepsis (EONS). The MCA, ICA, and vertebral artery (VA) have been studied; one study has shown nearly 100% sensitivity and diagnostic accuracy of measuring PI in VA.[2] Also, higher PSV has also been observed in these major vessels.[31]

Late-onset sepsis (LONS): Only a single study has specifically evaluated the effect of CBF in cases with LONS, demonstrating an increased incidence of RI (>0.82) in ACA in culture-proven sepsis, suggesting decreased flow.[35]

The variability in CBF data in LONS and EONS might be due to the extent of adversely disturbed autoregulatory cerebral circulation, where a critical drop of blood pressure in sepsis may have been transmitted directly to the cerebral vascular bed, leading to cerebral hypoperfusion.[36,37]

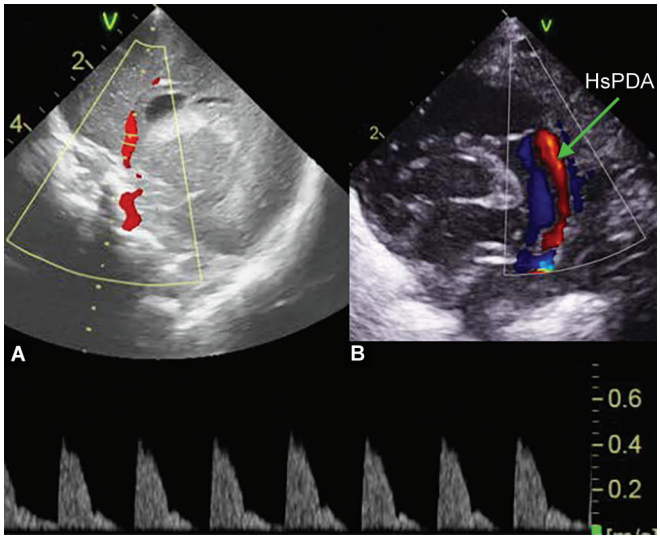
Shock

Abrupt changes in cardiac output and blood pressure can affect cerebral circulation with changes that are tenfold greater than in the systemic blood vessels.[38] Cerebral blood flow also falls with lower systemic blood pressure. In preterm infants with severe shock (MAP <10th centile), changes are clearly evident in MCA flow as low end-diastolic velocity and peak-systolic velocity.[6] This is further reaffirmed by the return of the cerebral blood flow velocity (CBFV) near to normal range on regaining systemic perfusion. In fact, marked beat-to-beat fluctuations and reversal of arterial flow in ACA represent troublesome findings and may precede IVH in preterm neonates.[7] With every 8 mL change in cardiac output (outside the range of 190–440 mL/kg/min), peak-systolic velocity changes by 1 cm, whereas this change is 1.5 cm with every mm Hg change in blood pressure (outside the range of 30–40 mm Hg) and changes in mean velocity by 0.15 cm/mm Hg.[39]

HsPDA

Altered CBFV secondary to HsPDA serves as a surrogate maker with both diagnostic and prognostic significance. An association between HsPDA with higher RI and lower MV in the ACA, MCA, and ICA has been observed in various clinical studies with a wide variability of RI ranging from 0.78 to 1.2 in cases with HsPDA to RI of 0.61–0.81 in preterm infants without a significant PDA representing an overlapping range.[13,37,40–42] These changes in CBF are attributed to the ductal steal phenomenon. Unaltered RI even in the presence of HsPDA could primarily be a result of unaltered autoregulation. Second, this might indicate that RI may not change when both diastolic and systolic flows are equally affected.

Bravo et al.[43] concluded that an RI of ≥0.74 in MCA serves as the best biomarker of moderate-to-large PDA (sensitivity 82%, specificity 72%, positive predictive value 50%, and negative predictive value 92%) when assessed 24 hours after termination of the ibuprofen course. In general, a Doppler assessment of the ACA showing a retrograde flow during diastole would suggest a significant ductal shunt (Fig. 13).



Figs 13A and B: 2D-Echo in a ductal view showing patent ductus arteriosus (red flow) with a cranial sagittal view pulsed-wave Doppler in the anterior cerebral artery showing increased RI with absent diastolic flow indicating HsPDA requiring treatment (HsPDA, hemodynamically significant patent *ductus arteriosus*)

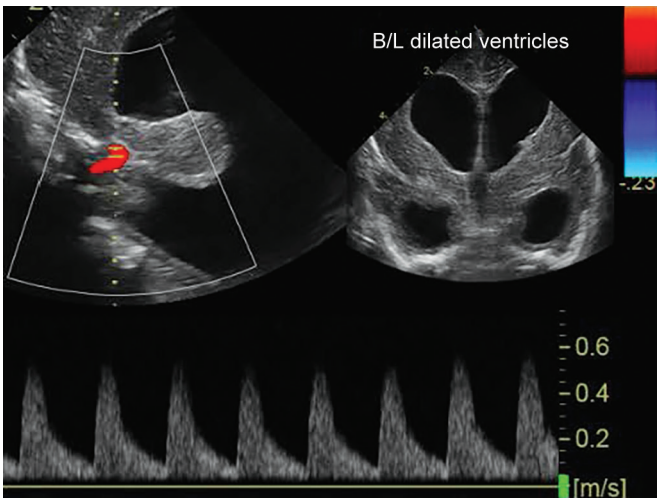


Fig. 14: Post-hemorrhagic hydrocephalus in a 1-month-old, premature infant born at a gestational age of 30 weeks. Coronal view showing posthemorrhagic hydrocephalus, PWD of ACA on sagittal view showing increased RI

ACA, anterior cerebral artery; PWD, pulse wave Doppler; RI, resistive index

Hydrocephalous

Correctly diagnosing hemodynamic alterations in progressive hydrocephalus could assist in determining the need for drainage, especially in cases of slowly progressive hydrocephalus. Baseline velocities and indices are usually normal in such cases. Hence, RI measured pre and post compression (delta RI = 1–19%) could represent disturbed cerebral compliance and need for shunting. Postsurgical blockage of a shunt could also be detected by the compression technique showing decreased or absent flow in cases of shunt occlusion (Fig. 14).[20]

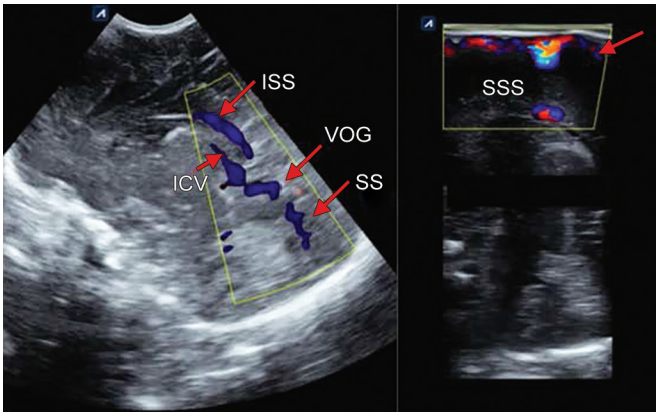


Fig. 15: Cerebral veins and sinuses seen through anterior fontanelle midsagittal view: (A) Inferior sagittal sinus (ISS), internal cerebral vein (ICV), vein of Galen (VOG), straight sinus (SS); (B) Superior sagittal sinus (SSS)

Assessment of Cerebral Vasculature, as in Arterial stroke, Cerebral Sino-venous Thrombosis (CSVT) Vascular Anomalies

Cerebral veins can be visualized in parasagittal planes through the anterior fontanelle with conventional pulsed-wave Doppler sample volume placed at a point midway along the length of the vein of Galen (mean velocity 5.5 ± 1.5 cm/s ± SD), along the straight sinus (behind the cerebellum), in the internal cerebral vein ([8.8 ± 3 cm/s ± SD] behind the 3rd ventricle), and superior sagittal sinus (16.5 ± 5 cm/s ± SD) (Fig. 15).

In a stable newborn, the cerebral venous pattern is typically characterized by continuous low-velocity flow. The four main venous flow patterns are band-like, sinusoid, intermittent, and reverse flow (Fig. 9). The first two patterns are seen in healthy neonates, and the last two prognosticate adverse outcomes in sick premature neonates, indicating reduced venous flow.

Doppler ultrasound can demonstrate partial or a total absence of flow in combination with partial or complete occlusion of the affected sinus(es) or vessel. Furthermore, it can depict associated brain lesions in the form of (late-onset) IVH and white matter injury. Previous retrospective studies reported only a moderate sensitivity of cranial ultrasound for the detection of CSVT. In the study by Berfelo et al., 37% of cases were diagnosed with cUS and 63% solely on MRI, and in another study, approximately half of CSVT was detected with cUS.[44,45]

In ultrasound evaluation, ischemic stroke appears as a wedge-shaped focal increase in echogenicity in the supply region of an artery, typically in the MCA. Doppler can be used to differentiate between complete occlusion and severe stenosis and the success of therapeutic measures can also be determined in the further course on the basis of the recanalization of vessels and the morphological consequences of stroke (cyst formation due to liquefactive necrosis).[46]

Vascular malformations such as the vein of Galen malformation, if untreated, showed pathologic high systolic (up to >1.0 m/s), very high diastolic velocities (up to >0.5 m/s), and low RI (<0.6) with statistically significant differences between the pre- and the post-embolization RI with pathologic low RI before and nearly normal RI after successful shunt reduction.[47]

Effect of Kangaroo Mother Care (KMC) on Cerebral Doppler

Kangaroo mother care is a multisensory stimulation strategy that is postulated to optimize CBF. Cerebral blood flow has shown a significantly sustained improvement after KMC in decreasing RI and increasing EDV and MV in MCA. This plausible improvement is attributed to the activation of slow-conducting unmyelinated afferents by tactile stimulation that activates the release of vasodilatory mediators in the cortex. The residual effects of KMC on the cerebral Doppler might provide an explanation of KMC on long-term neurodevelopment.[48,49]

Intrauterine Growth Restriction (IUGR)

Basu et al.[50] observed decreased PSV and higher RI in all cerebral vessels in IUGR neonates as compared with their appropriately grown counterparts and attributed it to higher venous hematocrit and lower CBFV. Cerebral Doppler in IUGR is a niche area of research that requires more rigorous evaluation; the results could help predict long-term neurodevelopment outcomes.

Brain Death

Transcranial Doppler examination may not be a consistently reliable marker of brain death and should not be used as a single modality for making these determinations. A reduced systolic antegrade flow followed by a diastolic retrograde flow and with the subsequent appearance of the characteristic short systolic spikes in ACA is reported in cases of brain death.[51]

Neurological Outcome

Low mean CBFV[52] and higher RI[53] reported in the first few days after birth in ACA are found to be associated with lower Griffith's score at 1–2 years of corrected age. Low CBFV is proposed to be a consequence, not a cause of brain injury, as the damaged brain does not grow and may require less blood flow.

Limitations

Being a nonhazardous low-cost, noninvasive modality, cranial Doppler has evoked interest. However, there are limitations as this is an operator-dependent tool with a learning curve; there is a need for expertise to derive a stable long-term signal to measure blood flow velocities. Inadequate acoustic windows in cases with overriding sutures and craniosynostosis can limit these evaluations. We need predictive tools that could combine CBF parameters with clinical parameters or validated clinical scores and can be used to postulate long-term neurological outcomes.

Conclusion

Doppler parameters can help in the near-accurate prediction of CBF, even though a single parameter might not be sufficient. Resistive index is the most user-friendly index that has a minimum inter-observer variability with no dependence on the angle of insonation. Elevated RI in the ACA and MCA can help assess a HsPDA. Doppler may also predict disability in infants with hypoxic brain damage before cooling. Further research is needed to assess its value in measuring vascular tone and/or shunts. A systematically conducted, adequately powered study is needed to evaluate the correlation of Doppler parameters to long-term neurodevelopment outcomes.

Orcid

Jyoti Patodia https://orcid.org/0000-0002-1722-409X

References

1. Meijler G, Steggerda SJ. Transcranial Doppler sonography in neonates. In: Neonatal Cranial Ultrasonography. In: Meijler G, Steggerda SJ (eds.). Springer Link; 2019. pp. 219–257.
2. Bada HS, Hajjar W, Chua C, et al. Noninvasive diagnosis of neonatal asphyxia and intraventricular hemorrhage by Doppler ultrasound. J Pediatr 1979;95(5 Pt 1):775–779. DOI: 10.1016/s0022-3476(79)80735-0.
3. Budohoski KP, Czosnyka M, Kirkpatrick PJ, et al. Clinical relevance of cerebral autoregulation following subarachnoid haemorrhage. Nat Rev Neurol 2013;9(3):152–163. DOI: 10.1038/nrneurol.2013.11.
4. Leon RL, Ortigoza EB, Ali N, et al. Cerebral blood flow monitoring in high-risk fetal and neonatal populations. Front Pediatr 2022;9:748345. DOI: 10.3389/fped.2021.748345.
5. Milona E, Karagianni P, Tsakalidis C, et al. Evaluation of cerebral oxygenation and perfusion in small for gestational age neonates and neurodevelopmental outcome at 24–36 months of age. J Perinat Med 2020;48(3):280–288. DOI: 10.1515/jpm-2019-0274.
6. Fenton AC, Woods KL, Evans DH, et al. Cerebrovascular carbon dioxide reactivity and failure of autoregulation in preterm infants. Arch Dis Child 1992;67(7 Spec No):835–839. DOI: 10.1136/adc.67.7_spec_no.835.
7. Bada HS, Korones SB, Kolni HW, et al. Partial plasma exchange transfusion improves cerebral hemodynamics in symptomatic neonatal polycythemia. Am J Med Sci 1986;291(3):157–163. DOI: 10.1097/00000441-198603000-00003.
8. Hamon I, Hascoet JM, Debbiche A, et al. Effects of fentanyl administration on general and cerebral haemodynamics in sick newborn infants. Acta Paediatr 1996;85(3):361–365. DOI: 10.1111/j.1651-2227.1996.tb14033.x.
9. Ichihashi K, Iino M, Eguchi Y, et al. Effect of head position to the cerebral arterial flow in neonates. Early Hum Dev 2002;69(1–2):35–46. DOI: 10.1016/s0378-3782(02)00037-3.
10. Buckley EM, Cook NM, Durduran T, et al. Cerebral hemodynamics in preterm infants during positional intervention measured with diffuse correlation spectroscopy and transcranial Doppler ultrasound. Opt Express 2009;17(15):12571–12581. DOI: 10.1364/oe.17.012571.
11. Duckrow RB. Decreased cerebral blood flow during acute hyperglycemia. Brain Res 1995;703(1–2):145–150. DOI: 10.1016/0006-8993(95)01077-7.
12. Pezzati M, Dani C, Biadaioli R, et al. Early postnatal Doppler assessment of cerebral blood flow velocity in healthy preterm and term infants. Dev Med Child Neurol 2002;44(11):745–752. DOI: 10.1017/s0012162201002870.
13. Ecury-Goossen GM, Raets MM, Camfferman FA, et al. Resistive indices of cerebral arteries in very preterm infants: Values throughout stay in the neonatal intensive care unit and impact of patent ductus arteriosus. Pediatr Radiol 2016;46(9):1291–1300. DOI: 10.1007/s00247-016-3615-x.
14. Archer LN, Evans DH, Levene MI. Doppler ultrasound examination of the anterior cerebral arteries of normal newborn infants: The effect of postnatal age. Early Hum Dev 1985;10(3–4):255–260. DOI: 10.1016/0378-3782(85)90056-8.
15. Allison JW, Faddis LA, Kinder DL, et al. Intracranial resistive index (RI) values in normal term infants during the first day of life. Pediatr Radiol 2000;30(9):618–620. DOI: 10.1007/s002470000286.
16. Bulas DI. Transcranial doppler: Applications in neonates and children. Ultrasound Clin 2009;4(4):533–551. DOI: 10.1016/j.cult.2009.11.001.
17. Horgan JG, Rumack CM, Hay T, et al. Absolute intracranial blood-flow velocities evaluated by duplex Doppler sonography in asymptomatic preterm and term neonates. AJR Am J Roentgenol 1989;152(5):1059–1064. DOI: 10.2214/ajr.152.5.1059.
18. Jarmund AH, Pedersen SA, Torp H, et al. A scoping review of cerebral Doppler arterial waveforms in infants. Ultrasound Med Biol 2023;49(4):919–936. DOI: 10.1016/j.ultrasmedbio.2022.12.007.
19. Seibert JJ, Chadduck WM, Seibert JJ, et al. Duplex pulsed Doppler US versus intracranial pressure in the neonate: Clinical and experimental studies. Radiology 1989;171(1):155–159. DOI: 10.1148/radiology.171.1.2648468.

20. Couture A, Veyrac C, Baud C, et al. Advanced cranial ultrasound: Transfontanellar Doppler imaging in neonates. Eur Radiol 2001;11(12):2399–2410. DOI: 10.1007/s00330-001-1150-z.
21. Menke J, Michel E, Rabe H, et al. Simultaneous influence of blood pressure, PCO2, and PO2 on cerebral blood flow velocity in preterm infants of less than 33 weeks' gestation. Pediatr Res 1993;34(2): 173–177. DOI: 10.1203/00006450-199308000-00014.
22. Camfferman FA, de Goederen R, Govaert P, et al. Diagnostic and predictive value of Doppler ultrasound for evaluation of the brain circulation in preterm infants: A systematic review. Pediatr Res 2020;87(Suppl 1):50–58. DOI: 10.1038/s41390-020-0777-x.
23. Van Bel F, Van de Bor M, Stijnen T, et al. Aetiological role of cerebral blood-flow alterations in development and extension of peri-intraventricular haemorrhage. Dev Med Child Neurol 1987;29(5):601–614. DOI: 10.1111/j.1469-8749.1987.tb08502.x.
24. Ikeda T, Amizuka T, Ito Y, et al. Changes in the perfusion waveform of the internal cerebral vein and intraventricular hemorrhage in the acute management of extremely low-birth-weight infants. Eur J Pediatr 2015;174(3):331–338. DOI: 10.1007/s00431-014-2396-1.
25. Okumura A, Toyota N, Hayakawa F, et al. Cerebral hemodynamics during early neonatal period in preterm infants with periventricular leukomalacia. Brain Dev 2002;24(7):693–697. DOI: 10.1016/s0387-7604(02)00083-9.
26. Fukuda S, Kato T, Kakita H, et al. Hemodynamics of the cerebral arteries of infants with periventricular leukomalacia. Pediatrics 2006;117(1):1–8. DOI: 10.1542/peds.2004-1719.
27. Natique KR, Das Y, Maxey MN, et al. Early use of transcranial doppler ultrasonography to stratify neonatal encephalopathy. Pediatr Neurol 2021;124:33–39. DOI: 10.1016/j.pediatrneurol.2021.07.004.
28. Archer LN, Levene MI, Evans DH. Cerebral artery Doppler ultrasonography for prediction of outcome after perinatal asphyxia. Lancet 1986;328(8516):1116–1118. DOI: 10.1016/s0140-6736(86)90528-3.
29. Liu J, Cao HY, Huang XH, et al. The pattern and early diagnostic value of Doppler ultrasound for neonatal hypoxic-ischemic encephalopathy. J Tropical Pediatr 2007;53(5):351–354. DOI: 10.1093/tropej/fmm046.
30. Rath C, Rao S, Suryawanshi P, et al. Does abnormal Doppler on cranial ultrasound predict disability in infants with hypoxic-ischaemic encephalopathy? A systematic review. Dev Med Child Neurol 2022;64(10):1202–1213. DOI: 10.1111/dmcn.15236.
31. Basu S, Dewangan S, Shukla RC, et al. Cerebral blood flow velocity in early-onset neonatal sepsis and its clinical significance. Eur J Pediatr 2012;171(6):901–909. DOI: 10.1007/s00431-011-1643-y.
32. Ratnaparkhi CR, Bayaskar MV, Dhok AP, et al. Utility of Doppler ultrasound in early-onset neonatal sepsis. Indian J Radiol Imaging 2020;30(1):52–58. DOI: 10.4103/ijri.IJRI_265_19.
33. Furukawa S, Sameshima H, Ikenoue T. Circulatory disturbances during the first postnatal 24 hours in extremely premature infants 25 weeks or less of gestation with histological fetal inflammation. J Obs Gynaecol Res 2008;34(1):27–33. DOI: 10.1111/j.1447-0756.2007.00678.x.
34. Pfister D, Schmidt B, Smielewski P, et al. Intracranial pressure in patients with sepsis. Acta Neurochir Suppl 2008;102:71–75. DOI: 10.1007/978-3-211-85578-2_14.
35. Yengkhom R, Suryawanshi P, Ingale S, et al. Resistive index in late-onset neonatal sepsis. J Neonatol 2018;32(4):93–97. DOI: https://doi.org/10.1177/0973217920901998.
36. Perlman JM, Hill A, Volpe JJ. The effect of patent ductus arteriosus on flow velocity in the anterior cerebral arteries: Ductal steal in the premature newborn infant. J Pediatr 1981;99(5):767–771. DOI: 10.1016/s0022-3476(81)80408-8.
37. Martin CG, Snider AR, Katz SM, et al. Abnormal cerebral blood flow patterns in preterm infants with a large patent ductus arteriosus. J Pediatr 1982;101(4):587–593. DOI: 10.1016/s0022-3476(82)80715-4.
38. Battisti O. Listening to the brain of the newborn by the Doppler Method; 2009. Available from: https://orbi.uliege.be ›/2268/15354/1.
39. Raju TN. Cranial Doppler applications in neonatal critical care. Critical care clinics 1992;8(1):93–111. DOI: https://doi.org/10.1016/S0749-0704(18)30269-0.
40. Vettukattil JJ. Pathophysiology of patent Ductus arteriosus in the preterm infant. Curr Pediatr Rev 2016;12(2):120–122. DOI: 10.2174/1573396312021605060022215.
41. Kupferschmid CH, Lang D, Pohlandt F. Sensitivity, specificity and predictive value of clinical findings, m-mode echocardiography and continuous-wave Doppler sonography in the diagnosis of symptomatic patent ductus arteriosus in preterm infants. Eur J Pediatr 1988;147(3):279–282. DOI: 10.1007/BF00442695.
42. Lipman B, Serwer GA, Brazy JE. Abnormal cerebral hemodynamics in preterm infants with patent ductus arteriosus. Pediatrics 1982;69(6):778–781. PMID: 7079043.
43. Bravo MC, Cabanas F, Riera J, et al. Randomised controlled clinical trial of standard versus echocardiographically guided ibuprofen treatment for patent ductus arteriosus in preterm infants: A pilot study. J Mater Fetal Neonatal Med 2014;27(9):904–909. DOI: 10.3109/14767058.2013.846312.
44. Berfelo FJ, Kersbergen KJ, Van Ommen CH, et al. Neonatal cerebral sinovenous thrombosis from symptom to outcome. Stroke 2010;41(7):1382–1388. DOI: 10.1161/STROKEAHA.110.583542.
45. Grunt S, Wingeier K, Wehrli E, et al. Cerebral sinus venous thrombosis in Swiss children. Dev Med Child Neurol 2010;52(12):1145–1150. DOI: 10.1111/j.1469-8749.2010.03722.x.
46. Deeg KH. Sonographic and Doppler sonographic diagnosis of neonatal ischemic stroke. Ultraschall Med 2017;38(4):360–376. DOI: 10.1055/s-0043-114409.
47. Meila D, Lisseck K, Jacobs C, et al. Cranial Doppler ultrasound in vein of Galen malformation. Neuroradiology 2015;57(2):211–219. DOI: 10.1007/s00234-014-1455-7.
48. Sahoo M, Dubey B, Vani K, et al. Changes in cerebral blood flow parameters among preterm 30–34 week neonates who are initiated on kangaroo mother care – A prospective analytical observational study. Early Hum Dev 2023;180:105764. DOI: 10.1016/j.earlhumdev.2023.105764.
49. Chaudhari AJ, Nimbalkar SM, Patel DV, et al. Effect of kangaroo mother care on cerebral hemodynamics in preterm neonates assessed by transcranial doppler sonography in middle cerebral artery. Indian Pediatr 2023;60(1):27–32. DOI: 10.1016/j.earlhumdev.2023.105764.
50. Basu S, Dewangan S, Barman S, et al. Postnatal changes in cerebral blood flow velocity in term intra-uterine growth-restricted neonates. Paediatr Int Child Health 2014;34(3):189–193. DOI: 10.1179/2046905514Y.0000000124.
51. McMenamin JB, Volpe JJ. Doppler ultrasonography in the determination of neonatal brain death. Ann Neurol 1983;14(3): 302–307. DOI: 10.1002/ana.410140308.
52. Ojala T, Kääpä P, Helenius H, et al. Low cerebral blood flow resistance in nonventilated preterm infants predicts poor neurologic outcome. Pediatr Crit Care Med 2004;5(3):264–268. DOI: 10.1097/01.pcc.0000112368.32965.45.
53. van Bel F, den Ouden L, van de Bor M, et al. Cerebral blood-flow velocity during the first week of life of preterm infants and neurodevelopment at two years. Dev Med Child Neurol 1989;31(3):320–328. DOI: 10.1111/j.1469-8749.1989.tb04001.x.

REVIEW ARTICLE

Lung Ultrasound as a Novel Tool to Assess the Severity and Management of Neonatal Pneumonia

Divya Durga[1], Usha Devi[2], Kirti Gupta[3], Akhil Maheshwari[4,5], Pradeep Suryawanshi[6]

Received on: 27 October 2023; *Accepted on*: 30 November 2023; *Published on*: 05 January 2024

Abstract

The use of lung ultrasound in neonatal intensive care units has greatly increased in recent years. Multicentric studies and meta-analyses have shown ultrasound as a tool with good sensitivity and specificity in the diagnosis of severe neonatal and childhood pneumonia. However, we still lack a standardized scoring system for neonatal pneumonia. In this paper, we propose a 5-grade lung ultrasound score (LUS) for increasing the severity of pneumonia, which indicates its progression and onset of associated complications. This bedside score using lung ultrasound will help in early detection, assessment of severity, and the need for timely administration of antibiotics.

Keywords: Alveolar-interstitial pattern, A-lines, Air bronchograms, Atelectasis, B-lines, Comet tail artifacts, Community-acquired, Consolidation, Dynamic air bronchograms, ESPNIC, Empyema, Hemithorax, Hepatization, High-frequency linear array transducer probe, Lobar lung collapse, Lung sliding, Lung ultrasound, Meconium aspiration syndrome, Micro-atelectasis, Neonatal pneumonia, Pleural line, Pneumonia, Point-of-care lung ultrasound, Respiratory distress syndrome, Static air bronchograms, Subpleural consolidations, Synpneumonic, Transient tachypnea, Transient tachypnea of newborn, Ventilator-associated pneumonia.

Newborn (2023): 10.5005/jp-journals-11002-0076

Key Points

- Neonatal respiratory pathologies are the leading cause of morbidity and mortality in neonatal intensive care units (NICUs).
- Point-of-care lung ultrasound (POCUS) is rapidly emerging as a useful bedside imaging modality for the diagnosis and management of various neonatal lung pathologies such as transient tachypnea, respiratory distress syndrome, pneumonia, atelectasis, and meconium aspiration.
- Multicentric studies and meta-analyses have shown ultrasound as a tool with good sensitivity and specificity in the diagnosis of severe neonatal and childhood pneumonia.
- In this paper, we propose a 5-grade lung ultrasound (LUS) score for increasing the severity of pneumonia, which indicates its progression and onset of associated complications.

Introduction

Point-of-care lung ultrasound (POCUS) is rapidly emerging as a useful bedside imaging modality for the diagnosis and management of various neonatal lung pathologies such as transient tachypnea, respiratory distress syndrome, pneumonia, atelectasis, and meconium aspiration syndrome.[1,2] It is highly sensitive and specific for the diagnosis of neonatal lung pathologies and can potentially add to radiography.[3,4] These real-time images can be obtained within a short time span that are convenient, cost-effective, have better sensitivity and specificity, are noninvasive, and have no radiation exposure to the neonate. The severity scores obtained with lung sonography can facilitate therapeutic decisions.[5–17]

Neonatal respiratory pathologies are the leading cause of morbidity and mortality in neonatal intensive care units (NICUs).[18,19] Several studies have shown that bedside lung ultrasound can help in the early diagnosis of lung pathologies such as micro-atelectasis and lobar collapse, and ventilator-associated pneumonia with higher sensitivity and specificity than radiography.[3,20] In developing countries, sepsis is still a major concern. Early diagnosis of pneumonia with high specificity and assessment of severity can guide therapy like early initiation of antibiotics, need for surfactant, initiation of assisted ventilation, and choice of ventilator settings for appropriate recruitment of lungs can be helpful. These steps can potentially reduce morbidity and mortality of critically ill neonates.[10–17]

[1]Department of Neonatology, Chengalpattu Medical College, Tamil Nadu, India

[2]Department of Neonatology, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

[3]Neonatal Intensive Care Unit, University College London Hospital, London, United Kingdom

[4]Global Newborn Society, Clarksville, Maryland, United States of America

[5]Department of Neonatology/Pediatrics, Louisiana State University-Shreveport, Shreveport, Louisiana, United States of America

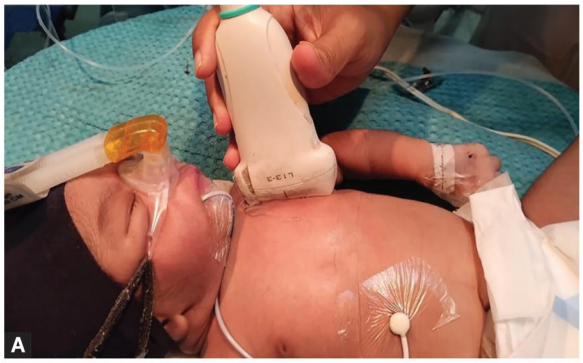
[6]Department of Neonatology, Bharati Vidyapeeth University Medical College Hospital and Research Center, Pune, Maharashtra, India

Corresponding Author: Pradeep Suryawanshi, Department of Neonatology, Bharati Vidyapeeth University Medical College Hospital and Research Center, Pune, Maharashtra, India, e-mail: drpradeepsuryawanshi@gmail.com

How to cite this article: Durga D, Devi U, Gupta K, *et al*. Lung Ultrasound as a Novel Tool to Assess the Severity and Management of Neonatal Pneumonia. Newborn 2023;2(4):291–296.

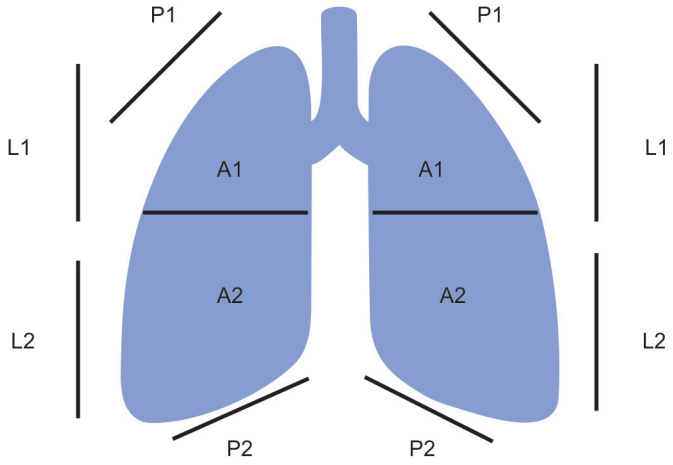
Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as 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 the Editor-in-Chief and his research group.



Chest	Zones	Boundaries
Anterior	A1	Upper: Clavicle; Lower: 4th rib; Medial: Sternal edge; Lateral: Defined by LUS image of the lung; beyond this is axilla
	A2	Upper: 4th rib; Lower: variable, depending on body habitus and defined by curtain sign in LUS and appearance of abdominal contents: Liver on the right side, bowel and spleen on the left; Medial: Sternal edge; Lateral: Anterior axillary line
Lateral	L1	Upper: Axilla; Lower: The axis of the 4th rib; Anterior: Anterior axillary line; Posterior: Posterior axillary line
	L2	Upper: The axis of the 4th rib; Lower: Variable, depending on body habitus; Anterior: Anterior axillary line; Posterior: Posterior axillary line
Posterior	P1	Upper: Defined by LUS image of the lung; Medial: Thoracic spine; Lateral: Medial border of scapula; Lower: Level of the inferior angle of the scapula
	P2	Upper: Level of the inferior angle of the scapula; Medial: Thoracic spine; Lateral: Posterior axillary line; Lower: Appearance of abdominal contents: Liver on the right side, bowel, and spleen on the left

B



Figs 1A and B: (A) Ultrasound examination of the neonatal lungs; photograph shows the probe placed in the left anterior upper quadrant; (B) Scanning regions in lung ultrasound. High-frequency linear array transducer probe shows anterior (A1, A2), lateral (L1, L2), and posterior (P1, P2) regions. Details of each region are shown in the third column. Graphic shows the location of these regions

In this paper, we propose to use POCUS and develop (a) a diagnostic scoring system for neonatal pneumonia based on lung ultrasound findings; (b) severity scores of pneumonia; and (c) list sonographic characteristics of atelectasis and various forms of neonatal pneumonia that may be congenital, ventilator-associated, or community-acquired.

Standard Operating Procedure for Lung Ultrasound

Lung ultrasound is founded on the principle of ultrasound waves' reflection by differential echogenicity of the pleura, lung tissue, and air.[21] The artifacts generated by the anatomical structures are useful in diagnosing various lung pathologies.[21] The neonatal lung is more suitable for visualization by lung ultrasound than older subjects because of the thin, relatively compliant chest wall, and the higher proportion of fluids.[20,22]

- For neonatal lung ultrasound, a high-frequency linear array transducer probe with a frequency greater than 10 MHz is suitable for examining each lung in 6 zones (Fig. 1A). Higher frequencies are useful in smaller babies.
- The transducer is held perpendicular to the ribs for imaging (Fig. 1B).
- Each hemithorax is divided into anterior, posterior, and lateral by anterior and posterior axillary lines, which are further divided into upper and lower in each region (Fig. 1A).
- In extremely low-birthweight (ELBW) and ventilated infants, in whom scanning the posterior lung fields is challenging, a 6-region approach can be useful where anterior upper, lower, and lateral areas of each hemithorax are visualized. The 6- or 12-region approach is used depending on the neonate's condition.[22]
- The standardization of guidelines for POCUS by the European Society of Paediatric and Neonatal Intensive Care (ESPNIC)[23] has reduced inter- and intra-observer variability and has also improved accuracy.

Normal Neonatal Lung Ultrasound Findings

A normal lung ultrasound image of neonate is composed of the following artifact lines generated by the pleura, air, and fluid present in the lung:

- Pleural line
- Lung sliding
- A-lines
- B-lines
- Comet-tail artifacts
- Air bronchograms
- Consolidation

Pleural line – A smooth, regular, thin, echogenic line seen just below the rib shadow, which moves with respiratory movements.

The normal thickness of a pleural line is typically generally less than 1 mm. The movement of the pleural line seen below the ribs synchronizes with respiratory movements, as seen in the lung ultrasound, and is called "lung sliding". This is a marker of a normal healthy lung (Fig. 2).

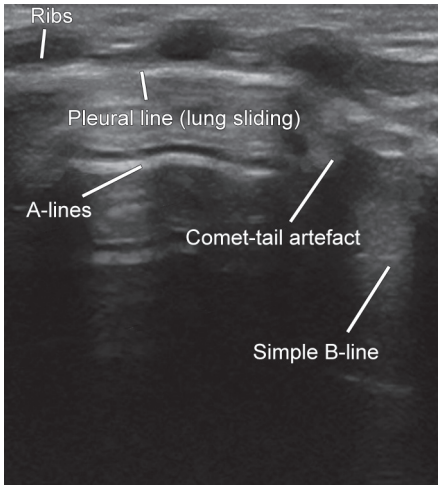
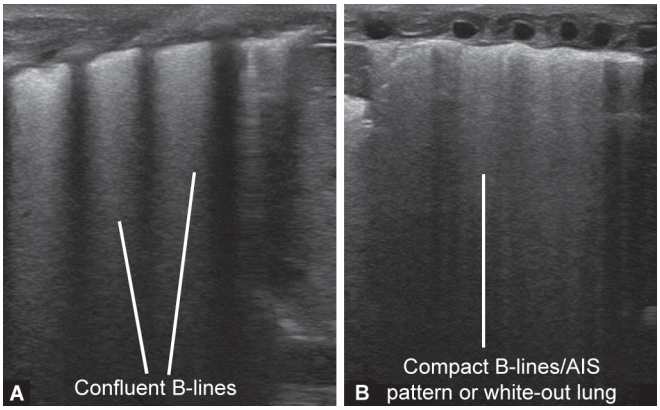


Fig. 2: Lung ultrasound findings with pleural lines, lung sliding, A-lines, and comet-tail artifacts. The name comet-tail artifact is used loosely to describe long, vertical, well-defined, hyperechoic, dynamic lines originating from the pleural line



Figs 3A and B: Lung ultrasound images demonstrating progression of simple to confluent B-lines and development of AIS pattern; (A) Confluent B-lines; (B) Compact B-lines/AIS pattern or white-out lung

A-lines – Horizontal echogenic lines that run parallel and below the pleural line. These are equidistant from each other. These artifacts are generated by the physiological air present within the lungs and signify a normally aerated lung that is sliding normally. The A-lines disappear in pathological conditions associated with fluid accumulation and/or inflammation, as seen in pneumonia, RDS, and transient tachypnea (Fig. 2).

B-lines – Vertical, laser-like, echogenic lines that may be 3–5 mm in depth. These artifacts are generated by fluid present in interstitial tissues of the lung. The B-lines increase in number (>3 B-lines) and may become confluent as the fluid levels increase. Confluent B-lines may merge with disappearing A-lines as the fluid enters into the alveoli and may form the so-called alveolar-interstitial pattern (AIS). In RDS, the rib shadows below the pleural line disappear, and B-lines merge. The lungs increasingly appear white-out on radiography. Ultrasound scans show considerable amounts of fluid (Fig. 3).

Comet-tail artifacts – Vertical echogenic lines that extend for a few mm and indicate small amounts of fluid seen in normal lungs (Fig. 2).

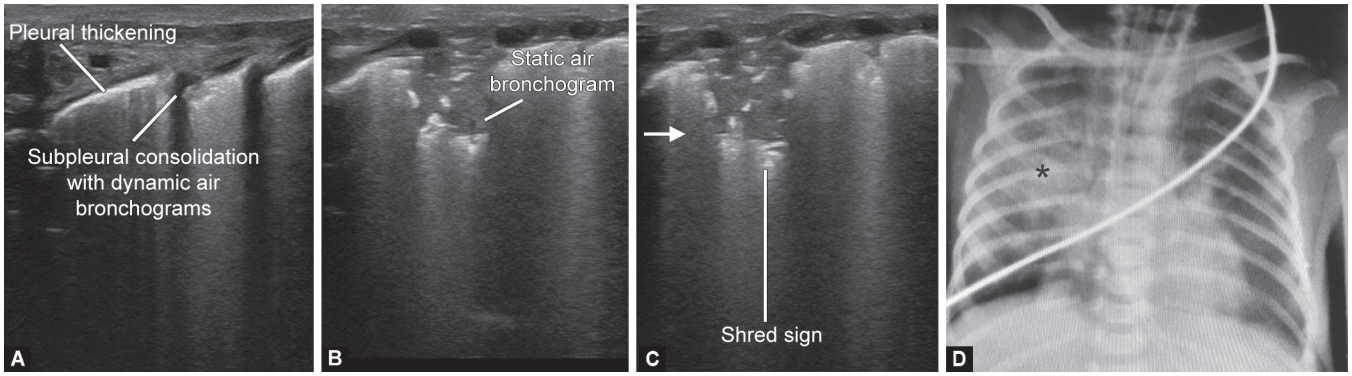
Air bronchograms – Echogenic dots that appear dense and flaky. When seen sliding along with the lung, these are called sliding, called dynamic air bronchograms. Other scans may show static air bronchograms. Dynamic air bronchograms are pathognomonic of RDS; pneumonia can show both static and dynamic air bronchograms (Fig. 3).

Consolidation – Lung tissue with density resembling that of liver showing air or fluid bronchograms (Fig. 4).

Shred sign – Seen in pneumonia along the margins of consolidated lung as tiny echogenic areas showing trapped air bronchograms (Fig. 4).

Patterns of POCUS Seen in Various Lung Pathologies

Pneumonia is an important cause of morbidity and mortality in infants.[18,19] Early and accurate diagnosis of pneumonia is crucial for efficient management and appropriate respiratory support. Bedside POCUS has emerged as a reliable and radiation-free tool for diagnosing neonatal pneumonia and helps in assessing the severity of the condition and guiding early initiation of antibiotics



Figs 4A to D: Image showing air bronchograms (static and dynamic), consolidation, and shred sign in neonates with pneumonia: (A) Marks show (i) pleural thickening and (ii) subpleural consolidations with dynamic air bronchograms; (B) Static air bronchograms; shred sign seen on; (C) Lung ultrasound and (D) In the corresponding chest X-ray. The shred sign shows irregular (shredded/fractal) margins between the consolidated and aerated lung

and respiratory support.[1,2,5–17] In pneumonia, lung ultrasound typically shows inflammatory changes such as varying levels of fluid in the interstitial tissue and alveoli, consolidation, air bronchograms, and effusion or empyema.[21] By providing real-time images, noninvasiveness, and quick results, lung ultrasound has proven advantageous over traditional chest X-rays.[3,4]

POCUS can show the following findings in neonatal pneumonia:

- *Pleural line abnormalities* – irregular or thickening of pleura.
- *Confluent or compact B-Lines* or interstitial syndrome pattern of fluid AIS can be seen.
- *Consolidation* of lung – "Hepatization" where the lung resembles the liver in echogenicity. Pleural sliding may be absent in the area of hepatization.
- *Shred sign* – the margins of lung hepatization seen as irregular echogenic spots that show areas of air entrapment within air bronchograms. Lungs affected by pneumonia show a shredded appearance, which has been named as the "shred" sign that is pathognomonic of pneumonia. Large areas of lung consolidation with irregular margins have nearly 100% sensitivity and specificity for the diagnosis of neonatal pneumonia.[1]
- *Air bronchograms* – dynamic or static air bronchograms can be seen in the involved areas.
- *Subpleural consolidations* can be seen as echogenic irregular areas.
- *Synpneumonic effusions*.
- *Empyema* – echogenic areas or septations can be seen in the pleural fluid collection (Table 1 and Figs 4 to 7).

Table 1: Scoring system for pneumonia by lung ultrasound

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

Ventilator-associated pneumonia – Lung ultrasound has emerged as a reliable tool for early diagnosis of ventilator-associated

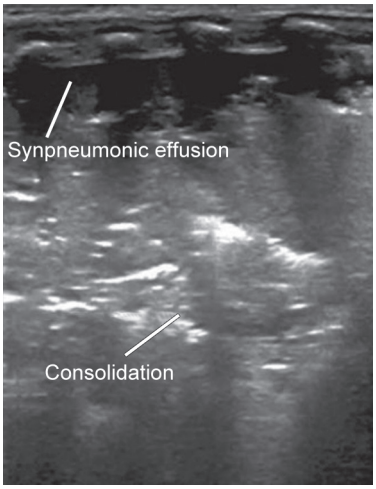


Fig. 5: Lung ultrasound image showing a synpneumonic effusion

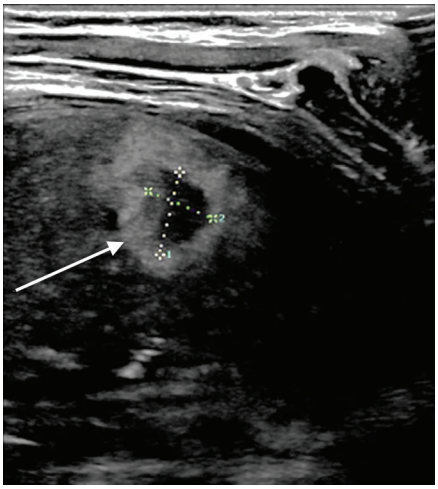
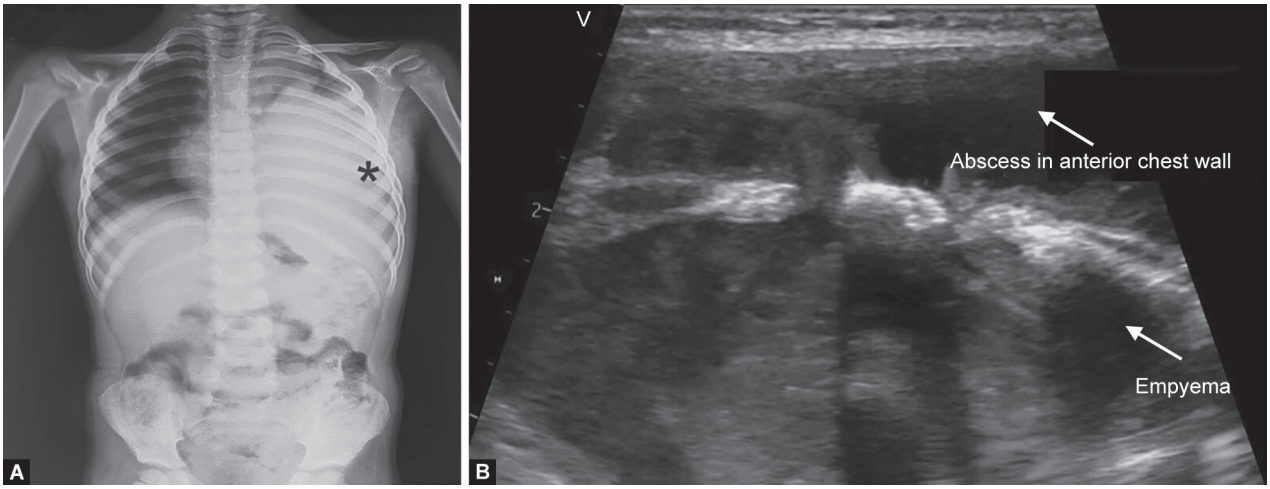
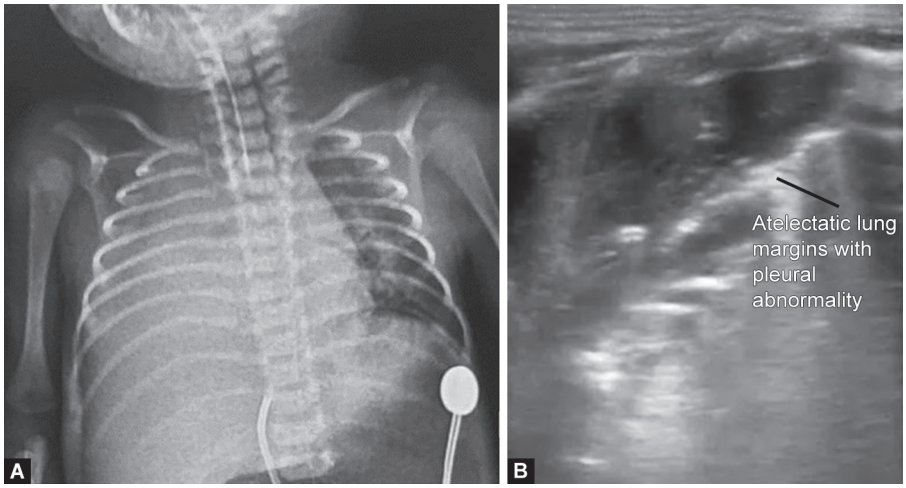


Fig. 6: Image of lung abscess on lung ultrasound (arrow)



Figs 7A and B: Lung ultrasound can add to the disease information obtained from radiography. (A) Chest X-ray showed a radiological opacity on the left side (asterisk); (B) Chest sonogram showed that the radiological opacity resulted from a combination of an abscess in the anterior chest wall and an underlying empyema



Figs 8A and B: A chest X-ray image and a lung ultrasound from an infant with atelectasis. A well-defined area of collapse can be seen on the right side. The ultrasound images added to the confidence in diagnosis by showing atelectatic lung margins with thickened visceral pleura

Table 2: Lung ultrasound findings in various types of neonatal pneumonia[3,18,19,23]

Congenital pneumonia	*Community-acquired pneumonia*	*Ventilator-associated pneumonia*	*Atelectasis*
Areas of consolidation, irregular margins	Hepatization with shred sign, irregular boundaries of lesion	Subpleural consolidations are hallmark	Well demarcated regular boundaries of consolidation. Shred sign less likely.
Interstitial syndrome pattern (AIS) with confluent or compact B-lines	Air bronchograms 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 less common	Synpneumonic effusion can be seen	Small areas of para-pneumonic effusion (less common)	Large effusion can be seen. Absent lung sliding in large area of involvement

pneumonia (VAP) in neonates. Hallmarks such as *subpleural consolidations* and *dynamic air bronchograms* correlate well with clinical VAP. Combining lung ultrasound with findings on cultures of endotracheal aspirates can enhance the accuracy of diagnosing VAP. Recent meta-analyses have shown that lung ultrasound outperforms chest X-ray in the diagnosis of community-acquired pneumonia with a sensitivity and specificity of 96% and 93%, respectively.[8] While there is no existing data on differentiating features of various types of pneumonia, such as congenital, community-acquired, or ventilation-associated pneumonia, certain features tend to be more prevalent in one form of pneumonia over the other.

Atelectasis – Accurate differentiation of respiratory distress in neonates due to infective causes like pneumonia from noninfective conditions such as respiratory distress syndrome (RDS) and lung collapse or atelectasis is crucial for proper management. Atelectasis, characterized by the resorption of air in alveoli, leading to the collapse of the affected lung area, can occur in various conditions such as RDS due to alveolar collapse, bronchial occlusion due to secretions as seen in pneumonia, or due to inadequate ventilation.[24]

Atelectatic regions can show well-defined, sizeable areas of consolidated lung: the margins may be clear and smooth, and air bronchograms can often be seen. Smaller areas of atelectasis may display indistinct margins, with pleural line abnormalities, loss of A-lines in affected areas, and the lung pulse sign. Areas with persistent lung collapse may not show any lung sliding (Fig. 8).[25] Lung ultrasound has been shown to detect areas of collapse missed by chest X-rays, it shows nearly 100% sensitivity for diagnosis of atelectasis compared with only 70% on chest X-rays.[26]

Limitations of Lung Ultrasound

There are some limitations to bedside lung ultrasound. Lesions containing entrapped air such as pulmonary bullae, empyema, and cystic changes such as in congenital pulmonary airway malformation (CPAM) and congenital lobar emphysema, may require further imaging such as computed tomography (CT) or magnetic resonance imaging (MRI) of the chest for definitive diagnosis.

Lung ultrasound cannot estimate the volume and types of pneumothorax, and pathologies such as pulmonary interstitial emphysema and those away from the pleura can be difficult to diagnose using this method.[27,28] Therefore, medical history and clinical correlation play a vital role in aiding the diagnosis of lung ultrasound findings, as this technique cannot differentiate infectious from noninfectious causes of consolidation and atelectasis.

To differentiate between types of pneumonia and to diagnose VAP, lung ultrasound may gain greater accuracy when combined

with cultures of tracheal aspirates or Gram stain. However, for neonates on high-frequency ventilation or with central lines, lung ultrasound of the posterior lung fields can be a challenge, necessitating bedside chest X-ray for diagnosis.

In conclusion, bedside lung ultrasound has emerged as an accurate, early, cost-effective, and radiation-free tool for diagnosing common neonatal conditions of respiratory distress requiring NICU admission. The lung ultrasound scoring system for pneumonia is a promising aid to therapy depending on the severity and monitoring of complications. We anticipate lung POCUS to gain considerable importance as an aid to radiography in NICUs.

References

1. Chen SW, Zhang MY, Liu J. Application of lung ultrasonography in the diagnosis of childhood lung diseases. Chin Med J 2015;128(19):2672–2678. DOI: 10.4103/0366-6999.166035.
2. Sharma D, Farahbakhsh N. Role of chest ultrasound in neonatal lung disease: A review of current evidences. J Matern Fetal Neonatal Med 2019;32(2):310–316. DOI: 10.1080/14767058.2017.1376317.
3. Chen S-W, Fu W, Liu J, et al. Routine application of lung ultrasonography in the neonatal intensive care unit. Medicine 2017;96(2):e5826. DOI: 10.1097/MD.0000000000005826.
4. Gao YQ, Qiu RX, Liu J, et al. Lung ultrasound completely replaced chest x-ray for diagnosing neonatal lung diseases: A 3-year clinical practice report from a neonatal intensive care unit in China. J Matern Fetal Neonatal Med 2022;35(18):3565–3572. DOI: 10.1080/14767058.2020.1830369.
5. Escourrou 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.
6. Cattarossi L, Copetti R, Poskurica B. Radiation exposure early in life can be reduced by lung ultrasound. Chest 2011;139(3):730–731. DOI: 10.1378/chest.10-2338.
7. Tandircioglu UA, Yigit S, Oguz B, et al. Lung ultrasonography decreases radiation exposure in newborns with respiratory distress: A retrospective cohort study. Eur J Pediatr 2022;181(3):1029–1035. DOI: 10.1007/s00431-021-04296-5.
8. Perri A, Sbordone A, Tirone C, et al. Early lung ultrasound score to predict noninvasive ventilation needing in neonates from 33 weeks of gestational age: A multicentric study. Pediatr Pulmonol 2022;57(9):2227–2236. DOI: 10.1002/ppul.26031.
9. Xi G, Dai J, Wang X, et al. Ultrasound performed shortly after birth can predict the respiratory support needs of late preterm and term infants: A diagnostic accuracy study. Pediatr Pulmonol 2021;56(7):2155–2163. DOI: 10.1002/ppul.
10. Rodríguez-Fanjul J, Balcells C, Aldecoa-Bilbao V, et al. Lung ultrasound as a predictor of mechanical ventilation in neonates older than 32 weeks. Neonatology 2016;110(3):198–203. DOI: 10.1159/000445932.
11. Raimondi F, Migliaro F, Sodano A, et al. Use of neonatal chest ultrasound to predict noninvasive ventilation failure. Pediatrics 2014;134(4):e1089–e1094. DOI: 10.1542/peds.2013-3924.
12. Abdelmawla M, Seleem W, Farooqui M, et al. Prediction of weaning readiness off nasal CPAP in preterm infants using point-of-care lung ultrasound. Pediatr Pulm 2022;57(9):2128–2135. DOI: 10.1002/ppul.26014.
13. Soliman RM, Elsayed Y, Said RN, et al. Prediction of extubation readiness using lung ultrasound in preterm infants. Pediatr Pulmonol 2021;56(7):2073–2080. DOI: 10.1002/ppul.25383.
14. Liang Z, Meng Q, You C, et al. Roles of lung ultrasound score in the extubation failure from mechanical ventilation among premature infants with neonatal respiratory distress syndrome. Front Pediatr 2021;9:709160. DOI: 10.3389/fped.2021.709160.
15. El Amrousy D, Elgendy M, Eltomey M, et al. Value of lung ultrasonography to predict weaning success in ventilated neonates. Pediatr Pulmonol 2020;55(9):2452–2456. DOI: 10.1002/ppul.24934.
16. Pierro M, Chioma R, Ciarmoli E, et al. Lung ultrasound guided pulmonary recruitment during mechanical ventilation in neonates: A case series. J Neonatal Perinatal Med 2022;15(2):357–365. DOI: 10.3233/NPM-210722.
17. Liu J, Xia RM, Ren XL, et al. The new application of point-of-care lung ultrasound in guiding or assisting neonatal severe lung disease treatment based on a case series. J Matern Fetal Neonatal Med 2020;33(23):3907–3915. DOI: 10.1080/14767058.2019.1590332.
18. Khemani RG, Smith LS, Zimmerman JJ, et al. Pediatric acute respiratory distress syndrome: Definition, incidence, and epidemiology. Pediatr Crit Care Med 2015;16(5 Suppl 1):S23–S40. DOI: 10.1097/PCC.0000000000000432.
19. Angus DC, Linde-Zwirble WT, Clermont G, et al. Epidemiology of neonatal respiratory failure in the United States: Projections from California and New York. Am J Respir Crit Care Med 2001;164(7):1154–1160. DOI: 10.1164/ajrccm.164.7.2012126.
20. Pereda MA, Chavez MA, Hooper-Miele CC, et al. Lung ultrasound for the diagnosis of pneumonia in children: A meta-analysis. Pediatrics 2015;135(4):714–722. DOI: 10.1542/peds.2014-2833.
21. O'Brien WD. Ultrasound-biophysics mechanisms. Prog Biophys Mol Biol 2007;93(1–3):212–255. DOI: 10.1016/j.pbiomolbio.2006.07.010.
22. Volpicelli G, Elbarbary M, Blaivas M, et al. International evidence-based recommendations for point-of-care lung ultrasound. Intensive Care Med 2012;38(4):577–591. DOI: 10.1007/s00134-012-2513-4.
23. Brusa G, Savoia M, Vergine M, et al. Neonatal lung sonography: Interobserver agreement between physician interpreters with varying levels of experience. J Ultrasound Med 2015;34(9):85–92. DOI: 10.7863/ultra.34.1.85.
24. Peroni DG, Boner AL. Atelectasis: Mechanisms, diagnosis and management. Paediatr Respir Rev 2000;1(3):274–278. DOI: 10.1053/prrv.2000.0059.
25. Caiulo VA, Gargani L, Caiulo S, et al. Usefulness of lung ultrasound in a newborn with pulmonary atelectasis. Pediatr Med Chir 2011;33(5–6):253–255. PMID: 22428435.
26. Liu J, Chen SW, Liu F, et al. The diagnosis of neonatal pulmonary atelectasis using lung ultrasonography. Chest 2015;147:1013–1019. DOI: 10.1378/chest.14-130.
27. Liu X, Si S, Guo Y, et al. Limitations of bedside lung ultrasound in neonatal lung diseases. Front Pediatr 2022;10:855958. DOI: 10.3389/fped.2022.855958.
28. Saraogi A. Lung ultrasound: Present and future. Lung India 2015;32(3):250–257. DOI: 10.4103/0970-2113.156245.

REVIEW ARTICLE

Bacteriophages

Srijan Singh[1], Gopal Nath[2], Akhil Maheshwari[3,4]

Received on: 23 October 2023; *Accepted on*: 24 November 2023; *Published on*: 05 January 2024

Abstract

Bacteriophages, viruses that invade bacterial cells, are the most abundant organisms in the biosphere. Phages include viruses with double-stranded DNA (most common), single-stranded DNA, single-stranded RNA, and double-stranded RNA (least common). Most virions (96%) are tailed; other types are cubic, filamentous, or pleomorphic. Phage genomes are diverse and pervasively mosaic owing to a high frequency of horizontal genetic exchange and recombinations. Phages may have lytic or lysogenic life cycles. They attach to specific bacteria and achieve killing by enzymes endolysins and holins, without affecting the commensal microflora because of host specificity. There is a constant "evolutionary arms race" which leads to competitive bacteria phage coevolution. Numerous diverse and sophisticated bacterial defense mechanisms are being developed to inhibit various stages of the phage life cycle. At the same time, phages have also evolved to overcome these bacterial defenses. Phage-based treatments are being developed where single phages, phage cocktails, phage-derived enzymes, phages in combination with antibiotics, and genetically modified phages might be useful. This can be useful in the treatment of infection with multidrug resistant (MDR) pathogens and also for biofilm removal.

Keywords: Abi-associated enzymes, Abortive infection, Adsorption block, Bacteriophage, Bacteriophage exclusion system, Biofilms, Bradley's classification, *Carjivirus communis*, Caudovirales, Chromosomal islands, Contractile tails, Cosmids, CrAssphage, CRISPER-cas bacterial immune system, Darwinian principles, Double-stranded DNA, Destruction of phage DNA after injection, Diversity-generating retroelements, dsDNA, Endolysin, Enterobacteria P4-like prophages, ESKAPE, Evolutionary arms race, Glucosyl-hydroxymethylcytosine, Helper proteins, Human phageome, Hydroxymethylcytosine, Infant, *Lactococcus* phage c2, Lit activator gol peptide, Long non-contractile tails, Lytic cycle, Lysogenic cycle, Metagenomics, Mosaicism, MS2 coat, *Mycoplasma* phage P1, Myoviridae, Neonate, Newborn, P2-like prophages, *Pasteurella* phage F108, Penetration block, Phage display, Phagemid, Phage coevolution, Phage cocktail, Phage terminase small subunit, Phage anti-restriction-induced system, Phage ecology, Podoviridae, Polyphage, Prophage, Prokaryote viruses, Prokaryotic argonautes, Pseudolysogenic cycle, Receptor, Receptor-binding proteins, Restriction-modification systems, RexAB system, Retrons, Short tails, Siphoviridae, ssRNA, Temperate phage, Toxin-antitoxin systems, Transduction, Virulent phage.

Newborn (2023): 10.5005/jp-journals-11002-0078

Key Points

- Bacteriophages are viruses that invade bacterial cells.
- These viruses can be double-stranded DNA (most common), single-stranded DNA, single-stranded RNA, and double-stranded RNA (least common).
- Most virions (96%) are tailed; other types are cubic, filamentous, or pleomorphic. Phage genomes are diverse and pervasively mosaic owing to a high frequency of horizontal genetic exchange and recombinations. Phages may have lytic or lysogenic life cycles.
- These virions attach to specific bacteria and achieve killing by enzymes endolysins and holins, without affecting the commensal microflora because of host specificity.
- Phage-based treatments of bacterial infections are being developed where single phages, phage cocktails, phage-derived enzymes, phages in combination with antibiotics, and genetically modified phages might be useful.

Introduction

Bacteriophages are viruses that invade bacterial cells. Bacteriophage is derived from "bacteria" and the Greek word *phagein*, signifying "to devor." The term "Prokaryote viruses" is a better term because it also includes viruses, mostly of hyperthermophiles, which do not resemble any conventional bacteriophages. They are the most abundant organisms in biosphere.[1]

Bacteriophages were the first type of viruses to be discovered. Many elemental discoveries of molecular biology—the proof that DNA was the molecule transmitting genetic information, the basic mechanisms of gene regulation, and the genetic code, were made using bacteriophages.[2] In 1972, Walter Fiers reported the first complete nucleotide sequence of a gene (gene encoding bacteriophage MS2 coat protein) and in 1976, of the viral genome of phage MS2.[3–5]

[1]Department of Neonatology, Kailash Hospital, Noida, Uttar Pradesh, India

[2]Department of Microbiology, Banaras Hindu University, Varanasi, Uttar Pradesh, India

[3]Global Newborn Society, Clarksville, Maryland, United States of America

[4]Department of Pediatrics/Neonatology, Louisiana State University, Shreveport, Louisiana, United States of America

Corresponding Author: Srijan Singh, Department of Neonatology, Kailash Hospital, Noida, Uttar Pradesh, India, Phone: +91 7011033174, e-mail: srijanstar89@gmail.com

How to cite this article: Singh S, Nath G, Maheshwari A. Bacteriophages. Newborn 2023;2(4):297–309.

Source of support: Nil

Conflict of interest: Dr Akhil Maheshwari is associated as 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 the Editor-in-Chief and his research group.

Table 1: Classification of prokaryote viruses[6]

Shape	*Nucleic acid*	*Family*	*Examples*
Tailed	dsDNA	*Myoviridae* (Tail contractile)	T4
		Siphoviridae (Tail long, non-contractile)	λ
		Podoviridae (Tail short)	T7
Polyhedral	ssDNA	*Microviridaae*	φX174
	dsDNA	*Corticoviridae, Tectiviridae,* SH1, STIV	PM2, PRD1
	ssRNA	*Leviviridae*	MS2
	dsRNA	*Cystoviridae*	φ6
Filamentous	ssDNA	*Inoviridae*	*MI3*
	dsDNA	*Lipothrixviridae, Rudiviridae*	*TTV1, SIRV-1*
Pleomorphic	dsDNA	*Plasmaviridae, Fuselloviridae, Guttaviridae, Ampullaviridae, Bicaudaviridae, Globuloviridae*	*L2, SSV-1, His-1*

In 1896, Ernest Hankin, a British bacteriologist noted marked antibacterial activity (against *Vibrio cholerae*) in the Ganga and Yamuna rivers in India, and attributed it to an unknown heat labile substance (with ability to pass through fine porcelain filters) and could limit the spread of cholera epidemics.[7]

Bacteriophages were discovered independently by FW Twort in 1915 and by Félix d'Herelle in 1917; hence, it is also known as BTwort-d'Herelle phenomenon or Bacteriophage phenomenon. D'Herelle's commercial laboratory in Paris synthesized five phage preparations against different bacteria, namely, Bacte´-coliphage, Bacte´-rhinophage, Bacte´-intesti-phage, Bacte´-pyo-phage, and Bacte´-staphy-phage, and were marketed by the French company L'Ore´al.[8]

Viral Structure

Bacteriophage genomes exhibit some unique features, namely, diversity, mosaicism, and differential gene mobility.

Diversity of the Bacteriophage Population

Phage genomes are enormously diverse,[9] accounting for 15% of all viruses with known unique sequences. There are around 750 unique, sequenced bacteriophage genomes, comprising of numerous virion morphologies and nucleic acid compositions. Most are double-stranded DNA (dsDNA) tailed phages (*Caudovirales*). There are around 50 each of completely sequenced RNA phages and single-stranded (ssDNA) phages, and 500 sequenced dsDNA tailed phages. Among the dsDNA tailed phages, 55% are morphologically *Siphoviridae* with long flexible non-contractile tails, 25% are *Myoviridae* with contractile tails and 20% are *Podoviridae* with short stubby tails.

Phage genome can be made up of 3,300 nucleotide ssRNA viruses of *Escherichia coli*[10] to the 500 kbp genome of *Bacillus megaterium* phage G. The smallest of the dsDNA tailed phages genomes are ~11.5 kbp such as *Mycoplasma* phage P1,[11] ~21 kbp such as *Lactococcus* phage c2,[12] and ~30 kbp *Pasteurella* phage F108[13] for the *Podoviridae, Siphoviridae*, and *Myoviridae* families, respectively. Further variation in size has been observed.

Phage viruses can contain dsDNA, which is most common ssDNA, ssRNA, and dsRNA. Most virions (96%) are tailed; other types are cubic, filamentous, or pleomorphic. The CFP categories include nearly 200 types, which comprise about 4% of all viruses. The term "cubic" refers to cubic symmetry and icosahedral shape. Table 1 denotes the classification of phages.

Phages with DNA genomes have the lowest per-nucleotide mutation rates and maximum genetic stability thereby having the ability to maintain larger genomes. Phages with ssRNA genomes have the maximum per-nucleotide mutations rates and smallest genomes.[14,15] Tailed phage genomes can range up to over 500 kb,[16,17] whereas the genomes of tailless phages are generally shorter than 15 kb.[15,18]

The term *polyphage* refers to filamentous phages, which are typically genomic multimers of phages with multiple viral particles encapsulated in the same set of coat proteins.[19]

Phage infectivity and stability is decided by the DNA content of capsid, which plays a role in evolutionary natural selection process, wherein, DNA is gained or lost to achieve virion stability. Being non-motile, phages require Brownian motion to reach their targets.[2]

Mosaicism

Mosaicism is the hallmark feature of bacteriophage genomes. Genome architecture of phages is pervasively mosaic, different segments have distinct evolutionary histories,[20] owing to horizontal genetic exchange. This is exemplified in Mycobacteriophages, where genetic assortment may be the result of repeated site-specific recombination and illegitimate recombination (genome acquisition of bacterial host genetic sequences).[21]

Differential Gene Mobility

Phage genomes are mosaic with a widespread non-homologous recombination across the genome with genes having differential mobility. Core gene recombinations that allow a more stable arrangement are selected during evolution; although promiscuous reassortments of the non-core genes are allowed.

Drivers of Bacteriophage Evolution

Phage evolution follows Darwinian principles. Their genomes bear the maximum genetic novelty among all organisms; with 80% of their encoded genes being unrelated to known proteins, and have unknown functions. This is relevant in the context of phage resistance; it helps in the evolution of phage variants that overcome resistant strains of bacteria. Simultaneously, there are host-mediated protection systems in play like restriction-modification,[22] CRISPR's,[23] tRNA cleavage,[24] and toxin-antitoxin systems.[25] In contrast, there are also phage-encoded mechanisms that enable variants with genome diversity to develop rapidly[26] along with genes that counteract host protection systems, such as

anti-restriction,[27] and RNA repair enzymes,[28] and those providing protection from other viruses.

There is a wide variability in phage length, ranging from 24 to 200 nm,[29] with T4 phages being largest measuring 200 nm in length and 80–100 nm in width. Phages may have an icosahedral shape or a shape with 20 sides and filaments.[30] Figure 1 shows the diagrammatic representation of a bacteriophage. The head or capsid is made up of protein subunits called protomers. The tail is composed of a hollow tube through which the nucleic acid passes into the bacterial host cell upon infection by a phage. Some phages do not possess a tail. The T4 phage has additional structures like baseplate and tail fibers attached to the tail which help attaching it to a bacterial host.[31] Table 2 summarizes the information of the major viral components. Figure 2 shows the transmission electron micrographs of phages.

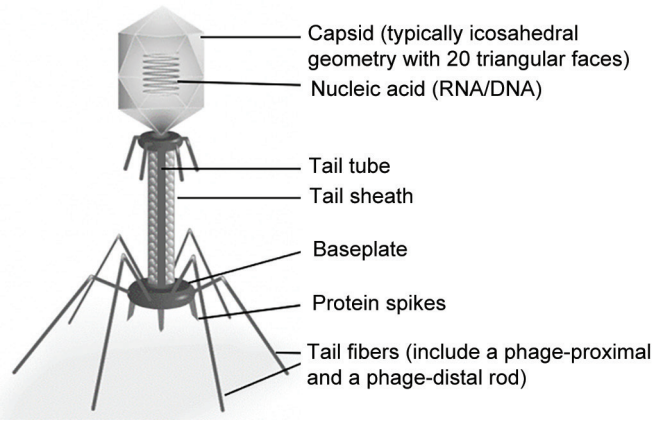


Fig. 1: Diagrammatic representation of a bacteriophage

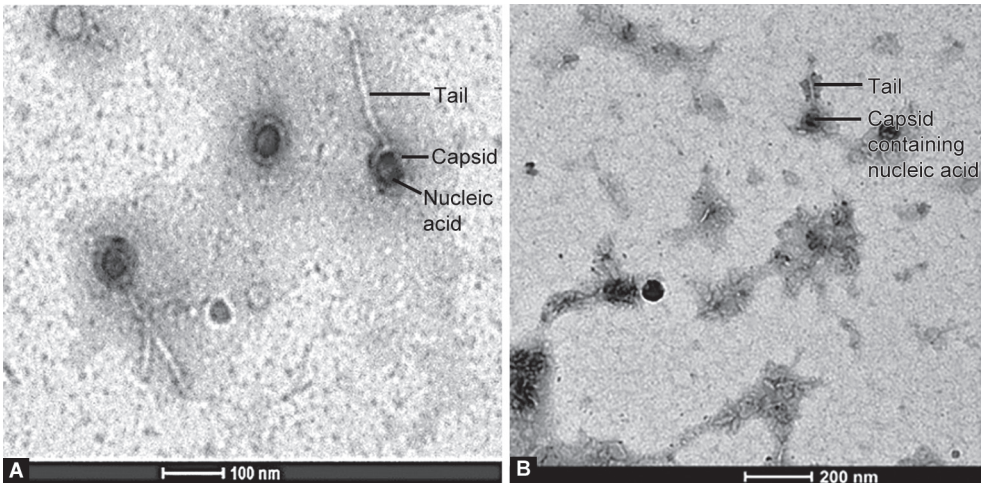
Table 2: Major structural components of bacteriophages

Structure	*Available information*
Lipid envelope	The lipoprotein envelope is derived from the nuclear membrane of an infected host cell[32] and covers the nucleocapsid. The only bacteriophages known to have a lipid envelope around their protein capsids are the members of the *Cystoviridae* family[33] which includes *Pseudomonas* phage phi6. It has three double-stranded RNA genome segments (S, M, and L) and a nucleocapsid surface shell formed by protein P8. Five viral membrane proteins are present—the major envelope protein P9, fusogenic protein P6, spike protein P3, putative holin protein P10, and minor membrane protein P13.[34]
Glycoproteins	Glycoproteins form membrane spikes on the virion surface. They are present in bacteriophages PM2, MX-1, P4, etc.[35–37]
Receptor-binding motifs	Receptor-binding motifs are involved in virion attachment to host cell surface receptors during the process of infection and endocytosis. Receptor-binding proteins (RBPs) of phages initiate infection of their bacterial host and act as the primary determinant for host specificity.[38]
Envelope protein	Envelope proteins facilitate attachment to cell surfaces and viral entry into the cells.[34,39,40]
Membrane protein	Holins and spanins are bacteriophage-encoded membrane proteins which control bacterial cell lysis in the final stage of their reproductive cycle. They accumulate in the membrane to disrupt the inner membrane and outer membrane of the bacteria.[41]
MHC or HLA proteins	Either not expressed or relevance unclear fetal/infantile disease.
Spike protein	Spike proteins allow phages to penetrate host cells and cause infection. Phage tail-spike proteins enable detection of pseudaminic acid-coated pathogenic bacteria and guide the development of antiglycan antibodies with cross-species antibacterial activity.
Surface tubules	Either not expressed or relevance unclear fetal/infantile disease.
Palisade layer	Either not expressed or relevance unclear fetal/infantile disease.
Viral tegument	Either not expressed or relevance unclear fetal/infantile disease.
Lateral bodies	Either not expressed or relevance unclear fetal/infantile disease.
Capsid	The capsid is a highly ordered proteinaceous structure which encloses the viral genomic RNA in nucleocapsid cores.[42]
Capsomeres	Structural subunits of the capsid are known as capsomeres and can be seen in electron micrographs. The head consists of numerous capsomeres with double-stranded DNA enclosed within.[43]
Core membrane	Either not expressed or relevance unclear fetal/infantile disease.
Protein core	Either not expressed or relevance unclear fetal/infantile disease.
Core fibrils	Either not expressed or relevance unclear fetal/infantile disease.
Matrix	Either not expressed or relevance unclear fetal/infantile disease.
Enzymes	Bacteriophage killing is achieved by two enzymes—endolysin and holin. Endolysins are phage-encoded peptidoglycan hydrolases produced at the end of the lytic cycle. Holin forms membrane lesions, through which endolysins cleave the peptidoglycan and cause host cell lysis and release of progeny phages.[44–46]
RNA elements	A transient double-stranded replicative RNA intermediate consisting of viral plus- and minus- strand RNAs is synthesized by a replicase complex formed by the non-structural proteins.[47,48]

(Contd...)

Table 2: *(Contd...)*

Structure	*Available information*
Nucleus	Bacteriophages φKZ and φPA3 encode a proteinaceous shell which assembles a nucleus-like structure to compartmentalize proteins and DNA during viral infection.[49]
Nucleosome	The genomes of some viruses (nuclear DNA viruses and retroviruses) requires evasion of host DNA damage recognition machinery. These genomes are organized into nucleosomes by annexing eukaryotic histones and the host nucleosome assembly machinery during latent and early lytic phase.
DNA	Bacteriophages may have ssDNA or dsDNA genome.[6]
RNA	Bacteriophages may have ssRNA or dsRNA genome.[6]
Genome-associated polyprotein	Protein-primed genome replication is a strategy to initiate DNA or RNA synthesis in linear genomes. Bacteriophage terminal proteins (TPs) are covalently attached to viral genomes to prime DNA replication. TPs are DNA-binding proteins and target phage genomes to the host nucleoid.[50]
DNA polymerase	Bacteriophage-encoded DNA polymerases are quite different from other known enzymes catalyzing DNA synthesis.[51] They can show activities unusual for the members of the family they belong to,[52] display unique structures,[53] or disobey canonical rules of base pairing during DNA synthesis adopted by all living organisms.[54]
RNA polymerase	The single subunit DNA-dependent RNA polymerase (RNAP), encoded by bacteriophage T7, is the prototype of a class of simple RNAPs (present in T3 and SP6, and the mitochondrial RNAPs). Phage-like RNAPs are related to other nucleotide polymerases such as DNA polymerases, RNA-dependent RNA polymerases, and RT.[55]
Reverse transcriptase	DNA synthesis is catalyzed by RNA-directed DNA polymerase (reverse transcriptase) in some phages.[56]
Head	Head is made of proteins and an inner core of nucleic acid. It is assembled as an empty capsid and thereafter, packaged with DNA by an ATP-dependent packaging machine, which binds to the same special pentameric vertex that is later occupied by the phage tail.
Baseplate	It is the most distal part of the tail of *Myoviridae* and *Siphoviridae,* and acts as a multiprotein molecular machine which binds to the host cell entry receptor that controls tail sheath contraction and initiates genome ejection by a change in the baseplate conformation.[57,58]
Integrase	Phage integrases are enzymes that mediate unidirectional site-specific recombination between two DNA recognition sequences, the phage attachment site, attP, and the bacterial attachment site, attB. They have two major families- the tyrosine recombinases and the serine recombinases, based on their mode of catalysis. Tyrosine family integrases, such as lambda integrase, utilize a catalytic tyrosine to mediate strand cleavage, recognize longer attP sequences, and require other proteins encoded by the phage or the host bacteria. Serine family integrases are larger, use a catalytic serine for strand cleavage, recognize shorter attP sequences, and do not require host cofactors.[59]
Tail	The phage tail is a complex, multiprotein structure that mediates attachment, digestion and penetration of the cell wall and genome ejection.[60,61]
Tail fiber	The host range of a phage is determined by phage tail fibers (or spikes), which mediate recognition and adsorption by specific bacteria.[62–64]
Neck	The neck of some bacteriophages (e/g T4) has "collar" and "whiskers", composed of fibritin molecules. Fibritin acts as a chaperone to facilitate attachment of long tail fibers to the virus during the assembly process.[65,66]



Figs 2A and B: Transmission electron micrographs of bacteriophages of the myoviridae family

Table 3: Differences between virulent and temperate phages

	Virulent phages	*Temperate phages*
Definition	Replicate by lytic cycle only	Replicate by both lytic and lysogenic cycle
Prophage stage	Not formed	Formed
Killing the host bacterium	Kill the host after each infection cycle	Do not kill the host immediately after infection
Transduction	Generalized transduction	Specialized transduction

Virus Taxonomy

Bradley's classification suggested six basic morphological types of tailed phages which were further divided on the basis of morphotypes (contractile tails, long and non-contractile tails, and short tails). Virus taxonomy is decided by the International Committee on the Taxonomy of Viruses (ICTV),[67] which published its first report in 1971. The Bacterial and Archaeal Viruses Subcommittee (BAVS) within ICTV holds the responsibility of classifying new prokaryotic viruses.[68] It considers numerous parameters like host range, physical characteristics (such as structure, capsid size, and shape), type of genomic material (single or double-stranded DNA or RNA), genome size, and resistance to organic solvents.[69]

In its report of March 2021–March 2022, Bacterial Viruses Subcommittee of the International Committee on Taxonomy of Viruses made drastic changes in bacteriophage taxonomy. Morphology-based families *Podoviridae*, *Siphoviridae*, and *Myoviridae* and the order *Caudovirales* were abolished, and a binomial system of nomenclature for species was established. One order, 22 families, 30 subfamilies, 321 genera, and 862 species were newly created.[70] The order *Caudovirales* was replaced by the class *Caudoviricetes* to encompass all tailed bacterial and archaeal viruses with icosahedral capsids and a dsDNA genome.

In 2020, ICTV endorsed binomial format for the naming of viruses, in which the genus name and a species epithet form a unique species name. For example, *Escherichia virus T4* was renamed *Tequatrovirus T4*, crAssphage was assigned to the species *Carjivirus communis*. The class *Caudoviricetes* now contains 14 families assigned to four orders, of which three orders include viruses infecting archaea.[71,72]

Mechanism of Proliferation

Bacteriophages exhibit host selectivity on account of requirement of specific receptors (lipopolysaccharides, teichoic acids, proteins, and flagella) on the bacterial surface for infection. External lipopolysaccharide (LPS) layer and embedded outer membrane proteins (OMPs) of Gram-negative bacteria are required for transport and diffusion of nutrients. These act as phage receptors, and are instrumental for adsorption of phage particles.[73] Teichoic acids of Gram-positive bacteria cell wall also work as phage receptors.[74,75]

There are different ways of insertion of genetic material in different phage groups. In the Myoviridae phage, after receptor recognition, the baseplate is attached with the bacterial surface by the flexing activity of tail fiber and inserts its genetic material by its tail contraction. Podoviridae phage is devoid of the tail part and inserts its genetic material after enzymatically degrading a portion of the bacterial cell membrane by small, tooth-like tail fibers.[73–75]

Replication of Phages

There are two types of life cycles of bacteriophages—lytic and lysogenic cycles.

Lytic Cycle

The lytic phase phages are also called as virulent phages. Following the multiplication of phages in the host, lysis and rupture of host bacteria occurs to release new phage particles. Host chromosome may be packed in to the capsid during phage replication which leads to horizontal gene transfer by transduction. Virulent phages may be used to counter the menace of antibiotic resistant pathogenic bacteria.

Lysogenic Cycle

Temperate phages undergo lysogenic phase in their life cycle wherein viral genetic material is integrated with the bacterial genome (called prophage), thereby ensuring continued replication of the viral genetic material without any fatal consequences to the infected host.[76] Phenotype of the infected bacteria changes; thereby bringing about its pathogenicity.[77,78] Prevention of lysogenic conversion can be done by hydrogen peroxide by production of a reactive oxygen species, glutathione, and overexpression of transcriptional repressors.[78–80]

Pseudolysogenic Cycle

Psuedolysogenic life cycle maybe shown by some phages. A phage enters a bacterial cell, does not integrate in a stable fashion and stays in this mode until there is a trigger for a lytic or lysogenic life cycle.[1]

Table 3 shows the differences between virulent and temperate phages.

Transduction

Phages are also a critical component of the human microbiome owing to being a mediator of genetic exchange between pathogenic and non-pathogenic bacteria.[81,82] Transduction is the transfer of genes from one bacterial strain to another by a bacteriophage and is of two types—generalized or specific. In "generalized" transduction, bacterial genomic DNA gets packaged inside phage capsids instead of phage genomic DNA during lytic cycle. This phage then infects a healthy host cell, integrates into its chromosome and alters the genome of the host as well as progeny. In "specialized" transduction, lysogenic phages excise parts of the bacterial DNA with their genome when initiating a lytic replication cycle. All progeny phages transduce the same bacterial gene to their hosts.[2]

Epidemiology

Bacteriophages are the most common organisms in the biosphere. Ganga water is an abundant natural source of diverse bacteriophages, which has the potential for the development of a phage bank, for the purpose of bacteriophage therapy in the future.[47]

Table 4: Antiphage defenses[83]

Bacterial defense mechanism	*Description*
Encounter blocks	Extracellular polymeric substances blocking virion approach to bacterial surfaces, e.g., capsules
Adsorption resistance (envelope-level resistance)	Binding failure due to absence of requisite receptor molecules on bacterial surfaces
Penetration blocks (exclusion; superinfection exclusion)	Blocks on phage movement during association with host, preventing entrance into host cytoplasm during adsorption
Immunity to superinfection (homoimmunity)	Blocks on phage replication due to recognition of specific phage-associated motifs
Abortive infection	Killing of phages but at cost of death of individual, phage-exposed bacteria
Restriction-modification	Generic features of organisms are targeted (recognition sequences found in DNA); equivalent host features are protected
Phage growth limitation system	Tagging of phages for elimination by clonally related cells
Opsonization	CRISPR phage resistance via acquisition of novel-to-host DNA sequence

Pathogenesis

Bacteriophage killing is attributed to two enzymes, endolysin and holin. Endolysins are phage-encoded peptidoglycan hydrolases, generated at the end of the lytic cycle. Holin forms membrane lesions, through which endolysins cleave the peptidoglycan, leading to host cell lysis and release of progeny phages.[47–49]

Phages do not affect the commensal microflora because of host specificity. Natural interactions among microbes boost phage generation which is exemplified by the Kumbh Mela community bath in Ganga river giving rise to high frequency of diversified bacteriophages.[47]

Interaction with mammalian hosts induces antiphages neutralizing antibodies, which peaks by the end of third week. Therefore, bacteriophages can be effectively used in acute cases with duration below 2 weeks. In chronic cases requiring prolonged therapy, phage cocktail of different antigenicities may be effective.[84]

Attachment and Penetration

Phages attach to specific receptors on the bacterial cell surface, including lipopolysaccharides, teichoic acids, proteins or flagella; hence, they infect only certain hosts which harbor those receptors. Attachment requires disintegration of the capsular outer layer of the hosts which is aided by polysaccharide-degrading enzymes of the virus. Phages are incapable of independent movements, hence require random encounters with the correct receptors in solution, such as blood, and lymphatic circulation.

Penetration of genetic material in the cell is done by a hypodermic syringe-like motion by Myovirus bacteriophages. Upon contact with the specific receptor, reversible binding is initiated wherein the tail fibers of bacteriophages flex to bring the baseplate closer to the surface of the cell. Thereafter, irreversible binding occurs by tail contraction to injecting genetic material through the bacterial membrane. Podoviruses enzymatically degrade bacterial membranes by small, tooth-like tail fibers to insert genetic material.[85]

Synthesis of Proteins and Nucleic Acid

After penetration of the genetic material, viral mRNA is translated to proteins by bacterial ribosomes. Bacterial RNA polymerase is modified by phages to preferentially transcribe viral mRNA, thereby disrupting synthesis of proteins and nucleic acids of the host. Virions are formed and assembled with the help of helper proteins.

Virion Assembly

Virion assembly is aided enzymatically by helper proteins. In T4 phage, morphogenesis starts initially with baseplate assembly followed by tails and subsequently, packaging of DNA in the head. An appropriate balance of morphogenetic proteins is mandatory for proper virion assembly which takes about 15 minutes.

Release of Virions

Virion release is followed by infection of a new host bacterium by the progeny phages. Tailed phages accomplish virion release by enzymatic breakdown of the peptidoglycan cell wall by endolysins, filamentous phages make the host secrete new virus particles and *Mycoplasma* phages achieve virion release by budding.

Communication

Bacteriophage Φ3T has been shown to signal other phages by Arbitrium protein.[86,87]

Antiphage Defense Mechanisms

Antiphage defense mechanisms have been developed by the prokaryotes to halt phage invasion and replication. The red queen hypothesis states that an organism must constantly evolve to maintain its relative fitness in the face of a predator.[88] There is a constant ''evolutionary arms race'' which leads to competitive bacteria phage coevolution.[89] Numerous diverse and sophisticated bacterial defense mechanisms are developed to inhibit every stage of the phage life cycle, whereas phages also evolve to overcome these bacterial defenses (Table 4).[90]

Adsorption Block

Bacteria develop strategies to decrease adsorption of phages to their specific bacterial receptors (protein, polysaccharide, or lipopolysaccharide LPS) by introducing mutations in the receptor structure and introducing physical barriers to camouflage receptors. This is seen in LamB, the phage lambda receptor, in *E. coli*-resistant cells.[91]

Penetration Block

Superinfection exclusion (SIE) is a bacterial defense in which intracellular phages, including prophages, block the infection of the same (homotypic SIE) or a different (heterotypic SIE) phage. Superinfection exclusion systems are phage-encoded

membrane-anchored or membrane-associated proteins and act to prevent phage DNA injection into bacterial hosts. They protect a lysogenized host from infection by other phages, thereby giving a strong selective advantage to the bacterium because they help protect the surrounding bacteria also since the infecting phage is rendered non-infectious after DNA ejection. The *E. coli* prophage HK97 confers both homotypic as well as heterotypic SIE thorough the expression of gp15.[92]

Restriction-modification Systems

Restriction-modification (R-M) systems include a restriction endonuclease (REase) and a cognate methyltransferase (MTase)[93] and halt replication and release of phages by destroying their DNA. The MTase methylates self-DNA at specific recognition sites, whereas foreign DNA stays unmodified, hence recognized by R-M REases and split into harmless fragments. The restriction endonuclease recognizes short DNA motifs of 4- to 8-base-pairs long, and cuts the phage DNA. These DNA motifs of the bacterial host are protected by methyltransferase to modify its own DNA to avoid recognition by the restriction enzyme.

Restriction-modification systems are of four types according to their subunit composition, recognition site, and mechanism of action.[93,94] Both type I and III systems translocate along DNA and cleave away from the recognition sites. Type II, used in molecular cloning, cleave within or near the recognition site. Type IV systems have a restriction endonuclease and lack a methylase, which cleaves only modified DNA. RM systems and DNA modifications exemplify an elaborate ''arms race'' between *E. coli* and phage T4. T4 contains hydroxymethylcytosine (HMC) in place of cytosine, inhibiting types I–III RM systems that recognize sites containing cytosine.[95] To counter this, *E. coli* uses McrBC, a type IV system specific for HMC-containing DNA.[96] In response, T4 can glycosylate its DNA, which impairs McrBC activity.[97] Against this, *E. coli* has evolved an additional type IV system, the GmrS-GmrD system, that can cleave glycosylated DNA.[98]

CRISPR-Cas bacterial immune systems[99] are present in approximately 50% of sequenced bacteria and 90% of sequenced archaea.[100] They are a part of adaptive immunity and provide resistance against invading phages[101] and plasmids.[102] Types I, II, and V use the crRNA guide to recognize the complementary target sequence in the DNA of the invader, known as the protospacer. In addition to this complementarity, cleavage requires the presence of a conserved protospacer-adjacent motif (PAM) in one flank of the target.[103–105] As a consequence of this targeting requirement, phages harboring mutations that eliminate the PAM or the complementarity between the protospacer and the crRNA can escape targeting.[106,107]

Prokaryotic Argonautes

Prokaryotic Argonautes (pAgos) are a bacterial innate defense mechanism in 9% of bacterial and 32% of archaeal genomes.[108] They are encoded within defense islands, regions enriched for phage resistance systems, and have undergone extensive horizontal gene transfer[109] defense mechanisms employed are DNA guided DNA silencing and RNA-guided DNA silencing. The apo form of pAgo can degrade invader DNA sequence and subsequent degradation products can serve as guide DNAs, which allows sequence-specific interference against the same target.[110,111]

Abortive Infection

Abi is a process to prevent the release of functional phage virions to prevent the predation of the surrounding clonal bacterial population at the expense of host cell survival. It is an altruistic action or a ''programmed cell death'' by disruption of essential cellular processes including translation, transcription, and replication or by inducing membrane leakage. Abi systems are encoded by mobile genetic elements, including prophages and plasmids[61] and are mechanistically diverse. The RexAB system in phage lambda protects lysogenized cells from infection by coliphages by inducing a loss of membrane potential, leading to decreased ATP levels.[112] Toxin-antitoxin (TA) systems have also been known to mediate abortive infection.[25]

Toxin-antitoxin Systems

TA gene pair consists of a toxin causing stress and antitoxin inhibiting the toxin's catalytic activity. They work as antiviral systems by disrupting phage life cycle and preventing virion release. Antitoxin is labile and requires continuous expression to remain at appropriate stoichiometric ratios with and neutralize the toxin.[113] Toxins possess catalytic activities, including DNase and RNase and can inhibit DNA replication, ATP synthesis and cell division machinery. There are six TA types, categorized based on the nature of the toxin and antitoxin (protein or RNA) and the mechanism of toxin neutralization.[113] TA systems have been implicated in phage defense, stress responses, plasmid maintenance, and persister cell formation.

Retrons

Retrons are bacterial genetic elements composed of a reverse transcriptase (RT) and a noncoding RNA (ncRNA) and protect against phage infection via abortive infection.[114,115] The Thoeris defense system is another mechanism which deploys a unique strategy for bacterial antiphage resistance via NAD^+degradation.[116]

Assembly Interference

Phage-inducible chromosomal islands (PICIs) are genetic elements that parasitize phages for replication and transmission.[117] PICIs are integrated into bacterial chromosomes and excise in the presence of a specific ''helper phage.'' PICI genomes are small (15 kb), encoding genes required for excision and integration, and a repressor that inhibits their expression. In *Staphylococcus aureus*, PICIs are named ''SaPIs'' (*S. aureus* pathogenicity islands), induced when their repressor, Stl, is sequestered away by an antirepressor expressed early during the helper phage lytic cycle.[118] They disseminate critical virulence factors.[119] They are induced to excise, replicate, and package themselves after infection by "helper" phages and allow the intracellular phage program to progress for the production of mature phage particles loaded with SaPI DNA.[120] Infected cell dies but phage reproduction is halted and SaPIs are spread to neighboring cells. They can also remodel the phage capsid proteins to generate small capsids that are tailored to the smaller SaPI genome and exclude the larger helper phage genome.[121,122] SaPIs encode phage packaging interference (Ppi) proteins, to block the phage terminase small subunit (required for recognition of phage DNA and initiation of packaging)[122] and also interrupt phage late gene activation, which is essential for phage packaging and cell lysis.[85]

Recently Discovered Antiphage Mechanisms

Enterobacteria P4- and P2-like prophages possess genetic hotspots that encode bacterial immune mechanisms[123] such as phage anti-restriction-induced system (PARIS). This system triggers an Abi response after sensing a phage-encoded anti-restriction

protein, Ocr, which inhibits R-M systems and bacteriophage exclusion (BREX).[123] The BREX system mediates methylation of a non-palindromic, six-nucleotide motif to achieve self-/non-self-discrimination,[124,125] aiding in the destruction of phage DNA after injection.

The DISARM mechanism[126] provides broad antiphage immunity by a novel RM-like mechanism that includes a methyltransferase modifying a five-nucleotide motif and a multicomponent restriction element to cleave phage DNA early in the phage life cycle.

Phage Counterattack Strategies

In response to diverse bacterial defenses, phages have developed novel counterattack mechanisms. To overcome variations in the bacterial cell surface receptors, phages vary their tropism through mutations in their RBPs. Receptor binding protein genes and genes mediating host recognition are prone to mutations, due to the activity of diversity-generating retroelements (DGRs).[127] These are prone to targeted mutation by exchange of two variable repeats by an error-prone RT.[128]

To overcome the barrier imposed by capsules and extracellular layers, some phages develop the ability to bind to these structures,[129] and degrade them using depolymerases. These enzymes may be either expressed as part of tail-spike or tail-fiber proteins or released in a soluble form following lysis of infected bacteria.[130]

Phages can counter RM systems in the following ways: (i) Removal of restriction sites from their genome (palindrome avoidance) to prevent recognition by REases;[131,132] (ii) modification of sequences recognized by REases (e.g. the glucosyl-hydroxymethylcytosine of T4 that is used instead of cytosine;[133] (iii) changing of distance and orientation of restriction sites to avoid restriction by REases;[134] (iv) occlusion of restriction sites with proteins;[135] (v) sequesteration of REases with proteins;[136] and (vi) acquisition of genes encoding an MTase that modifies the phage genome.[137]

CRISPR-Cas systems can be evaded[138] by acquisition of point mutations or deletions in the PAM sequences.[139] Anti-CRISPR (Acr) proteins avert recruitment of the crRNA-Cas complex to target DNA by binding the complex or occluding the PAM sequence, glucosylation of phage genetic material, or by inhibiting the endonuclease domain to prevent cleavage.[140]

Phages avert toxin–antitoxin systems by inhibiting the protease that degrades the antitoxin, expressing their own antitoxin analogue[141,142] or mutations in genes involved in the metabolism of nucleic acids.[143]

Mutations in phage genes which operate Abi-associated enzymes, such as the Lit activator Gol peptide in T4, obstructs Abi mechanism.[144] The Ocr protein of phage T7 sabotages the BREX system by binding the methyltransferase BrxX.[145]

Bacteriophage-host Symbiosis

Temperate phages integrate their genetic material as extrachromosomal episomes or as prophage during a lysogenic cycle which helps in achieving antibiotic resistance through the transfer or introduction of antibiotic resistance genes protecting hosts from phagocytosis, protecting hosts from secondary infection through superinfection exclusion, increasing host pathogenicity, or enhancing bacterial metabolism or growth. It provides selective advantages to bacteria with simultaneous passive replication of the phage genome.

Phage population in humans is known as human phageome (DHP) and comprises of healthy gut phageome (HGP) and the DHP. The active phageome of a healthy human (i.e., actively replicating as opposed to nonreplicating, integrated prophage) has been estimated to comprise dozens to thousands of different viruses.[146] Bacteriophages are found in 62% of healthy individuals but are reduced in those with inflammatory bowel diseases and in elderly. CrAssphages, most common phages in the human intestine, may be vertically transmitted with unique crAssphage clusters present in all humans.

Phage Ecology

Phage ecology is the study of the interaction of bacteriophages with their environment. It encompasses the study of impact of the abiotic environment on organisms (organismal ecology), impact of individuals of the same species on each other (population ecology), interactions between different species (community ecology), and the interaction and impact of biotic entities on abiotic aspects of environments (ecosystem ecology).[14]

Metagenomics is a concept that refers to the examination of genetic data from environmental samples in order to identify microbial communities.[147] A high-throughput sequence (HTS) based functional metagenomics technique is useful for identifying resistance mechanisms[148,149] and genomic content.

Phage Therapy

Phage-based treatments comprise of phage therapy with single phages or phage cocktails, phage-derived enzymes, phages in combination with antibiotics, and genetically modified phages.[150] These focus on lytic phages because they lack integrases and other enzymes involved in horizontal gene transfer.

Lysins and depolymerases are phage degradation enzymes useful in the removal of biofilms. Lysins are peptidoglycan hydrolases with a bactericidal effect on susceptible bacteria. They break peptidoglycan bonds, degrading the bacterial cell wall and biofilm structure, hence useful for gram-positive bacteria. Depolymerases are enzymes which degrade extracellular substances of encapsulated bacteria to reach phage receptors. They can degrade the chains of capsular polysaccharides, exopolysaccharides, and O-polysaccharides from lipopolysaccharides and peptidoglycan, which are components of the biofilm matrix. Bacteriophage recombinant lytic proteins (lysins and depolymerases) can be used as enzybiotics.[151]

The most common cause of opportunistic infections is the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) group of organisms, most of which are MDR isolates.[152] A sub-lethal dose of antibiotics can stimulate phage virulence under certain conditions known as phage-antibiotic synergy (PAS).[153,154] The combination of phage with antibiotics could have a variety of outcomes, including additive, synergistic, ineffective, or antagonistic effects.[155,156] Principi et al.[157] suggested that bacteriophages reduce the minimum inhibitory concentration of AR bacteria to the level of sensitive bacteria.

Temperate phages with phenotypic characteristics useful for biofilm removal can be turned into virulent phages by genetic engineering. Lytic phages are used to destroy bacteria and temperate phages are useful in delivering programmable DNA

Table 5: Definitions associated with bacteriophages

Virulent phage	Phages which display only lytic cycles (no chronic or lysogenic cycles)
Temperate phage	Phages which can undergo either virion productive or lysogenic cycles
Induction	Viral infection changes from a lysogenic cycle to a productive cycle
Chronic cycle/infection	Non- bactericidal phage infection, where virions are produced and continuously released
Productive cycle/infection	Virus reproduction with production of virion particles
Prophage	Phage genome that replicates with its host cell while not generating virion progeny
Cryptic prophage	Prophage that has mutationally lost its ability to enter a virion-productive cycle
Lysogen	Bacterial cell that harbors at least one prophage
Polylysogen	Bacterial cell that harbors more than one prophage
Transduction	Virion-mediated transfer of bacterial DNA to new bacteria either with associated temperate phage genome (specialized transduction) or not in association with phage genome (generalized transduction)
Virome	Metagenomic sequence of viral communities
Phagemid	Phasmid or phagemids are DNA-based cloning vectors possessing both bacteriophages and plasmid traits
Polyphage	Genomic multimers of phages with multiple viral particles being encapsulated in the same set of coat proteins, seen in filamentous phages
Superinfection exclusion	A bacterial defense in which intracellular phages, including prophages, block the infection of the same (homotypic SIE) or a different (heterotypic SIE) phage
Phage display	A molecular biology technique in which phage genomes are modified resulting in coat proteins of the assembled virions being fused to other proteins of interest, thereby displaying them to the external milieu
Phage ecology	Study of interaction of bacteriophages with their environment
Metagenomics	Examination of genetic data from environmental samples to identify microbial communities
Human phageome	Phage population in humans. It includes the "healthy gut phageome" (HGP) and the "diseased human phageome" (DHP)
Restriction-modification (R-M) systems	Restriction endonuclease (REase) and a cognate methyltransferase (MTase) present in some bacteria that halt replication and release of phages by destroying their DNA

nucleases associated with CRISPR to reverse antibiotic resistance and destruction of plasmids that confer antibiotic resistance.

Phage display is a molecular biology technique in which phage genomes are modified resulting in coat proteins of the assembled virions being fused to other proteins of interest, thereby displaying them to the external milieu. It aids isolation of proteins with desired affinity, specificity, stability, and enzymatic activity.[158]

Phagemid

Phasmid or phagemids are DNA-based cloning vectors possessing both bacteriophages and plasmid traits. They carry an origin of replication obtained from the bacteriophage along with origin of replication of the plasmid. Phagemids have the ability to be packaged into the capsid of bacteriophage as they contain a genetic sequence for packaging, unlike plasmids. Hence, they are useful in phage display and generating templates for site directed mutagenesis.[159] Cosmids are hybrid plasmids containing a lambda phage cos sequence and have 37–52 kb of DNA. They are used as cloning vectors and can be used to build genomic libraries.[160]

Table 5 presents the common definitions associated with bacteriophages.

Future Directions

Currently, no framework[161] exists to define phage as a medicinal product for human use, although institutes in Georgia, Poland, provide customized phage cocktails to chronically ill patients after exhaustion of conventional treatment options.[162] The need of the hour is good quality placebo-controlled randomized controlled trials along with a monitoring system for bacterial resistance to phages.[163–165] Good manufacturing practice level facilities are necessary for phage production and research[166,167] in order to ensure phage stability and effectiveness.

References

1. Clokie MR, Millard AD, Letarov AV, et al. Phages in nature. Bacteriophage 2011;1(1):31–45. DOI: 10.4161/bact.1.1.14942.
2. Kasman LM, Porter LD. Bacteriophages. Brenner's Encyclopedia of Genetics: Second Edition. Published online September 26, 2022: 280–283. DOI: 10.1016/B978-0-12-374984-0.00131-5.
3. Fiers W, Contreras R, Duerinck F, et al. Complete nucleotide sequence of bacteriophage MS2 RNA: primary and secondary structure of the replicase gene. Nature 1976;260(5551):500–507. DOI: 10.1038/260500A0.
4. Jou WM, Haegeman G, Ysebaert M, et al. Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein. Nature 1972;237(5350):82–88. DOI: 10.1038/237082A0.
5. Heather JM, Chain B. The sequence of sequencers: the history of sequencing DNA. Genomics 2016;107(1):1–8. DOI: 10.1016/j.ygeno.2015.11.003.
6. Ackermann HW. Phage classification and characterization. Methods Mol Biol 2009;501:127–140. DOI: 10.1007/978-1-60327-164-6_13.

7. Hankin ME. The bactericidal action of the waters of the Jamuna and Ganges rivers on Cholera microbes. Ann Inst Pasteur 10:511–523 (1896) 2011;1(3):117–126. DOI: 10.4161/BACT.1.3.16736.
8. Sulakvelidze A, Alavidze Z, Morris J. Bacteriophage therapy. Antimicrob Agents Chemother 2001;45(3):649–659. DOI: 10.1128/AAC.45.3.649–659.2001.
9. Hatfull GF. Bacteriophage genomics. Curr Opin Microbiol 2008;11(5):447–453. DOI: 10.1016/J.MIB.2008.09.004.
10. Friedman SD, Genthner FJ, Gentry J, et al. Gene mapping and phylogenetic analysis of the complete genome from 30 single-stranded RNA male-specific coliphages (family Leviviridae). J Virol 2009;83(21):11233–11243. DOI: 10.1128/JVI.01308-09.
11. Tu AHT, Voelker LRL, Shen X, et al. Complete nucleotide sequence of the mycoplasma virus P1 genome. Plasmid 2001;45(2):122–126. DOI: 10.1006/PLAS.2000.1501.
12. Lubbers MW, Waterfield NR, Beresford TPJ, et al. Sequencing and analysis of the prolate-headed lactococcal bacteriophage c2 genome and identification of the structural genes. Appl Environ Microbiol 1995;61(12):4348–4356. DOI: 10.1128/AEM.61.12.4348-4356.1995.
13. Campoy S, Aranda J, Àlvarez G, et al. Isolation and sequencing of a temperate transducing phage for Pasteurella multocida. Appl Environ Microbiol 2006;72(5):3154–3160. DOI: 10.1128/AEM.72.5.3154-3160.2006.
14. Introduction to phage evolutionary biology. Paul Turner Lab. Accessed on: July 31, 2023. Available from: https://turnerlab.yale.edu/introduction-phage-evolutionary-biology.
15. Abedon ST. Phage evolution and ecology. Adv Appl Microbiol 2009;67(C):1–45. DOI: 10.1016/S0065-2164(08)01001-0.
16. Seaman PF, Day MJ. Isolation and characterization of a bacteriophage with an unusually large genome from the Great Salt Plains National Wildlife Refuge, Oklahoma, USA. FEMS Microbiol Ecol 2007;60(1):1–13. DOI: 10.1111/J.1574-6941.2006.00277.X.
17. Serwer P, Hayes SJ, Thomas JA, et al. Propagating the missing bacteriophages: a large bacteriophage in a new class. Virol J 2007;4(1):1–5. DOI: 10.1186/1743-422X-4-21/FIGURES/1.
18. Fauquet CM, Fargette D. International Committee on Taxonomy of Viruses and the 3,142 unassigned species. Virol J 2005;2:64. DOI: 10.1186/1743-422X-2-64.
19. Lopez J, Webster RE. Morphogenesis of filamentous bacteriophage f1: Orientation of extrusion and production of polyphage. Virology 1983;127(1):177–193. DOI: 10.1016/0042-6822(83)90382-3.
20. Hendrix RW, Smith MCM, Burns RN, et al. Evolutionary relationships among diverse bacteriophages and prophages: all the world's a phage. Proc Natl Acad Sci USA 1999;96(5):2192–2197. DOI: 10.1073/PNAS.96.5.2192.
21. Pope WH, Jacobs-Sera D, Russel DA, et al. Expanding the diversity of mycobacteriophages: insights into genome architecture and evolution. PLoS One 2011;6(1): e16329. DOI: 10.1371/JOURNAL.PONE.0016329.
22. King G, Murray NE. Restriction enzymes in cells, not eppendorfs. Trends Microbiol 1994;2(12):465–469. DOI: 10.1016/0966-842X(94)90649-1.
23. Deveau H, Garneau JE, Moineau S. CRISPR/Cas system and its role in phage-bacteria interactions. Annu Rev Microbiol 2010;64:475–493. DOI: 10.1146/ANNUREV.MICRO.112408.134123.
24. Amitsur M, Levitz R, Kaufmann G. Bacteriophage T4 anticodon nuclease, polynucleotide kinase and RNA ligase reprocess the host lysine tRNA. EMBO J 1987;6(8):2499–2503. DOI: 10.1002/J.1460-2075.1987.TB02532.X.
25. Fineran PC, Blower TR, Foulds IJ, et al. The phage abortive infection system, toxin, functions as a protein-RNA toxin-antitoxin pair. Proc Natl Acad Sci U S A 2009;106(3):894–899. DOI: 10.1073/PNAS.0808832106.
26. Medhekar B, Miller JF. Diversity-generating retroelements. Curr Opin Microbiol 2007;10(4):388–395. DOI: 10.1016/J.MIB.2007.06.004.
27. McMahon SA, Roberts GA, Johnson KA, et al. Extensive DNA mimicry by the ArdA anti-restriction protein and its role in the spread of antibiotic resistance. Nucleic Acids Res 2009;37(15):4887–4897. DOI: 10.1093/NAR/GKP478.
28. Zhu H, Yin S, Shuman S. Characterization of polynucleotide kinase/phosphatase enzymes from Mycobacteriophages omega and Cjw1 and vibriophage KVP40. J Biol Chem 2004;279(25):26358–26369. DOI: 10.1074/JBC.M403200200.
29. Bacteriophage. Accessed on: July 24, 2023. Available from: https://www.microbiologybook.org/mayer/phage.htm.
30. Bacteriophage. Accessed on: July 11, 2023. Available from: https://www.microbiologybook.org/mayer/phage.htm.
31. Li W, Caberoy NB. New perspective for phage display as an efficient and versatile technology of functional proteomics. Appl Microbiol Biotechnol 2010;85(4):909. DOI: 10.1007/S00253-009-2277-0.
32. Laurinavičius S, Käkelä R, Bamford DH, et al. The origin of phospholipids of the enveloped bacteriophage phi6. Virology 2004;326(1):182–190. DOI: 10.1016/J.VIROL.2004.05.021.
33. Van Etten JL, Vidaver AK, Koski RK, et al. Base composition and hybridization studies of the three double-stranded RNA segments of bacteriophage phi 6. J Virol 1974;13(6):1254–1262. DOI: 10.1128/JVI.13.6.1254-1262.1974.
34. Lyytinen OL, Starkova D, Poranen MM. Microbial production of lipid-protein vesicles using enveloped bacteriophage phi6. Microb Cell Fact 2019;18(1):1–9. DOI: 10.1186/S12934-019-1079-Z/FIGURES/3.
35. Tsopanakis C, Parish JH. Bacteriophage MX-1: Properties of the phage and its structural proteins. J General Virol 1976;30(1):99–112. DOI: 10.1099/0022-1317-30-1-99/CITE/REFWORKS.
36. sid – Glycoprotein 3 - Enterobacteria phage P4 (Bacteriophage P4) | UniProtKB | UniProt. Accessed on: July 30, 2023. Available from: https://www.uniprot.org/uniprotkb/P05461/entry.
37. Camerini-Otero RD, Datta A, Franklin RM. Structure and synthesis of a lipid-containing bacteriophage: XI. Studies on the structural glycoprotein of the virus particle. Virology 1972;49(2):522–536. DOI: 10.1016/0042-6822(72)90504-1.
38. Boeckaerts D, Stock M, De Baets B, et al. Identification of phage receptor-binding protein sequences with hidden Markov models and an extreme gradient boosting classifier. Viruses 2022; 14(6):1329. DOI: 10.3390/V14061329/S1.
39. Zhai L, Anderson D, Bruckner E, et al. Novel expression of coat proteins from thermophilic bacteriophage ΦIN93 and evaluation for assembly into virus-like particles. Protein Expr Purif 2021;187:105932. DOI: 10.1016/J.PEP.2021.105932.
40. Mäntynen S, Sundberg LR, Oksanen HM, et al. Half a century of research on membrane-containing bacteriophages: Bringing new concepts to modern virology. Viruses 2019;11(1):76. DOI: 10.3390/V11010076.
41. Abeysekera GS, Love MJ, Manners SH, et al. Bacteriophage-encoded lethal membrane disruptors: Advances in understanding and potential applications. Front Microbiol 2022;13:1044143. DOI: 10.3389/FMICB.2022.1044143/BIBTEX.
42. Carmody CM, Goddard JM, Nugen SR. Bacteriophage capsid modification by genetic and chemical methods. Bioconjug Chem 2021;32(3):466. DOI: 10.1021/ACS.BIOCONJCHEM.1C00018.
43. Eiserling FA, Boy De La Tour E. Capsomeres and other structures observed on some bacteriophages. Pathobiology 1965;28(1):175–180. DOI: 10.1159/000161628.
44. Mishra RR, Nath G. Detection of bacteriophages against escape group of nosocomial pathogens from Ganga river water during community bath at various rituals: Since 2013–2019. J Appl Pharmaceut Sci Res 2020;3(1):17–21. DOI: 10.31069/japsr.v3i1.5.
45. Young R. Bacteriophage holins: Deadly diversity. J Mol Microbiol Biotechnol 2002;4(1):21–36. PMID: 11763969.
46. Borysowski J, Weber-Dąbrowska B, Górski A. Bacteriophage endolysins as a novel class of antibacterial agents. Exp Biol Med (Maywood) 2006;231(4):366–377. DOI: 10.1177/1535370206231 00402.
47. Henkin TM. Posttranscriptional Regulation. Encyclopedia of Microbiology, 3rd Edition. Published online January 1, 2009:342–356. DOI: 10.1016/B978-012373944-5.00086-9.
48. Mills DR, Priano C, Merz PA, et al. Q beta RNA bacteriophage: Mapping cis-acting elements within an RNA genome. J Virol 1990;64(8):3872. DOI: 10.1128/JVI.64.8.3872-3881.1990.

49. Chaikeeratisak V, Nguyen K, Egan ME, et al. The phage nucleus and tubulin spindle are conserved among large Pseudomonas phages. Cell Rep 2017;20(7):1563. DOI: 10.1016/J.CELREP.2017.07.064.
50. Redrejo-Rodríguez M, Salas M. Multiple roles of genome-attached bacteriophage terminal proteins. Virology 2014;468–470:322–329. DOI: 10.1016/J.VIROL.2014.08.003.
51. Morcinek-Orłowska J, Zdrojewska K, Węgrzyn A. Bacteriophage-encoded DNA polymerases—beyond the traditional view of polymerase activities. Int J Mol Sci 2022;23(2). DOI: 10.3390/IJMS23020635.
52. Berjón-Otero M, Villar L, De Vega M, et al. DNA polymerase from temperate phage Bam35 is endowed with processive polymerization and abasic sites translesion synthesis capacity. Proc Natl Acad Sci U S A 2015;112(27):E3476–E3484. DOI: 10.1073/PNAS. 1510280112.
53. Chen X, Su S, Chen Y, et al. Structural studies reveal a ring-shaped architecture of deep-sea vent phage NrS-1 polymerase. Nucleic Acids Res 2020;48(6):3343–3355. DOI: 10.1093/NAR/GKAA071.
54. Pezo V, Jaziri F, Bourguignon PY, et al. Noncanonical DNA polymerization by aminoadenine-based siphoviruses. Science 2021;372(6541):520–524. DOI: 10.1126/SCIENCE.ABE6542.
55. McAllister WT, Raskin CA. The phage RNA polymerases are related to DNA polymerases and reverse transcriptases. Mol Microbiol 1993;10(1):1–6. DOI: 10.1111/J.1365-2958.1993.TB00897.X.
56. Frolova LY, Metelyev VG, Ratmanova KI, et al. Reverse transcription of phage RNA and its fragment directed by synthetic heteropolymeric primers. Nucleic Acids Res 1977;4(7):2145. DOI: 10.1093/NAR/4.7.2145.
57. Yap ML, Rossmann MG. Structure and function of bacteriophage T4. Future Microbiol 2014;9(12):1319. DOI: 10.2217/FMB.14.91.
58. Gruidl ME, Chen TC, Gargano S, et al. Two bacteriophage T4 baseplate genes (25 and 26) and the DNA repair GeneuvsY belong to spatially and temporally overlapping transcription units. Virology 1991;184(1):359–369. DOI: 10.1016/0042-6822(91)90852-3.
59. Groth AC, Calos MP. Phage integrases: Biology and applications. J Mol Biol 2004;335(3):667–678. DOI: 10.1016/j.jmb.2003.09.082.
60. Hofer U. The sting is in the phage's tail. Nature Rev Microbiol 2016;14(8):477–477. DOI: 10.1038/nrmicro.2016.97.
61. Aksyuk AA, Leiman PG, Kurochkina LP, et al. The tail sheath structure of bacteriophage T4: A molecular machine for infecting bacteria. EMBO J 2009;28(7):821. DOI: 10.1038/EMBOJ.2009.36.
62. Taslem Mourosi J, Awe A, Guo W, et al. Understanding bacteriophage tail fiber interaction with host surface receptor: the key "blueprint" for reprogramming phage host range. Int J Mol Sci 2022;23(20):12146. DOI: 10.3390/IJMS232012146.
63. Hyman P, van Raaij M. Bacteriophage T4 long tail fiber domains. Biophys Rev 2018;10(2):463. DOI: 10.1007/S12551-017-0348-5.
64. Leiman PG, Arisaka F, Van Raaij MJ, et al. Morphogenesis of the T4 tail and tail fibers. Virol J 2010;7(1):1–28. DOI: 10.1186/1743-422X-7-355/FIGURES/16.
65. Fokine A, Zhang Z, Kanamaru S, et al. The molecular architecture of the bacteriophage T4 Neck. J Mol Biol 2013;425(10):1731. DOI: 10.1016/J.JMB.2013.02.012.
66. Akhter T, Zhao L, Kohda A, et al. The neck of bacteriophage T4 is a ring-like structure formed by a hetero-oligomer of gp13 and gp14. Biochim Biophys Acta 2007;1774(8):1036–1043. DOI: 10.1016/J.BBAPAP.2007.05.011.
67. Lefkowitz EJ, Dempsey DM, Hendrickson RC, et al. Virus taxonomy: The database of the International Committee on Taxonomy of Viruses (ICTV). Nucleic Acids Res 2018;46(D1):D708–D717. DOI: 10.1093/NAR/GKX.932.
68. Adriaenssens EM, Rodney Brister J. How to name and classify your phage: An informal guide. Viruses 2017;9(4):70. DOI: 10.3390/V.9040070.
69. Murphy FA. International Committee on Taxonomy of Viruses., International Union of Microbiological Societies. Virology Division. Virus taxonomy: Classification and nomenclature of viruses: Sixth report of the International Committee on Taxonomy of Viruses. Published online 1995:586.
70. Turner D, Shkoporov AN, Lood C, et al. Abolishment of morphology-based taxa and change to binomial species names: 2022 taxonomy update of the ICTV bacterial viruses subcommittee. Arch Virol 2023;168(2). DOI: 10.1007/S00705-022-05694-2.
71. Liu Y, Demina TA, Roux S, et al. Diversity, taxonomy, and evolution of archaeal viruses of the class Caudoviricetes. PLoS biology 2021;19(11):e3001442. DOI: 10.1371/journal.pbio.3001442.
72. Walker PJ, Siddell SG, Lefkowitz EJ, et al. Recent changes to virus taxonomy ratified by the International Committee on Taxonomy of Viruses (2022). Arch Virol 2022;167(11):2429–2440. DOI: 10.1007/s00705-022-05516-5.
73. Rakhuba D V, Kolomiets EI, Dey ES, et al. Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. Pol J Microbiol 2010;59(3):145–155. PMID: 21033576.
74. Brown S, Santa Maria JP, Walker S. Wall teichoic acids of gram-positive bacteria. Annu Rev Microbiol 2013;67:313–336. DOI: 10.1146/annurev-micro-092412-155620.
75. León M, Bastías R. Virulence reduction in bacteriophage resistant bacteria. Front Microbiol 2015;6(APR):135678. DOI: 10.3389/fmicb.2015.00343.
76. Inal JM. Phage therapy: A reappraisal of bacteriophages as antibiotics. Arch Immunol Ther Exp (Warsz) 2003;51(4):237–244. PMID: 12956433.
77. Brüssow H, Canchaya C, Hardt WD. Phages and the evolution of bacterial pathogens: From genomic rearrangements to lysogenic conversion. Microbiol Mol Biol Rev 2004;68(3):560–602. DOI: 10.1128/MMBR.68.3.560-602.2004.
78. Keen EC. Paradigms of pathogenesis: Targeting the mobile genetic elements of disease. Front Cell Infect Microbiol 2012;2:161. DOI: 10.3389/fcimb.2012.00161.
79. Wagner PL, Acheson DWK, Waldor MK. Human neutrophils and their products induce Shiga toxin production by enterohemorrhagic Escherichia coli. Infect Immun 2001;69(3):1934–1937. DOI: 10.1128/IAI.69.3.1934-1937.2001.
80. Ptashne M. Lambda's switch: Lessons from a module swap. Curr Biol 2006;16(12): R459-R462. DOI: 10.1016/j.cub.2006.05.037.
81. Watson BNJ, Staals RHJ, Fineran PC. CRISPR-Cas-mediated phage resistance enhances horizontal gene transfer by transduction. mBio 2018;9(1):e02406–e02417. DOI: 10.1128/MBIO.02406-17.
82. Boyd EF. Bacteriophage-encoded bacterial virulence factors and phage-pathogenicity island interactions. Adv Virus Res 2012;82:91–118. DOI: 10.1016/B978-0-12-394621-8.00014-5.
83. Abedon ST. Bacterial 'immunity' against bacteriophages. Bacteriophage 2012;2(1):50. DOI: 10.4161/BACT.18609.
84. Archana A, Patel PS, Kumar R, et al. Neutralizing antibody response against subcutaneously injected bacteriophages in rabbit model. Virus Dis 2021;32(1):38–45. DOI: 10.1007/S13337-021-00673-8.
85. São-José C. Engineering of phage-derived lytic enzymes: Improving their potential as antimicrobials. Antibiotics 2018;7(2):29. DOI: 10.3390/antibiotics7020029.
86. Callaway E. Do you speak virus? Phages caught sending chemical messages. Nature. Published online 2020. DOI: 10.1038/NATURE.2017.21313.
87. Erez Z, Steinberger-Levy I, Shamir M, et al. Communication between viruses guides lysis-lysogeny decisions. Nature 2017;541(7638):488. DOI: 10.1038/NATURE21049.
88. McLaughlin RN, Malik HS, Levine JD, et al. Genetic conflicts: The usual suspects and beyond. J Exp Biol 2017;220(1):6–17. DOI: 10.1242/JEB.148148.
89. Svircev A, Roach D, Castle A. Framing the future with bacteriophages in agriculture. Viruses 2018;10(5):218. DOI: 10.3390/V10050218.
90. Rostøl JT, Marraffini L. (Ph)ighting phages: How bacteria resist their parasites. Cell Host Microbe 2019;25(2):184–194. DOI: 10.1016/J.CHOM.2019.01.009.
91. Clément JM, Lepouce E, Marchal C, et al. Genetic study of a membrane protein: DNA sequence alterations due to 17 lamB point mutations affecting adsorption of phage lambda. EMBO J 1983;2(1):77–80. DOI: 10.1002/J.1460-2075.1983.TB01384.X.

92. Cumby N, Edwards AM, Davidson AR, et al. The bacteriophage HK97 gp15 moron element encodes a novel superinfection exclusion protein. J Bacteriol 2012;194(18):5012–5019. DOI: 10.1128/JB.00843-12/ASSET/4105D23E-A668-44C7-8B0F-440FF1AB86C5/ASSETS/GRAPHIC/ZJB9990918960006.JPEG.
93. Tock MR, Dryden DTF. The biology of restriction and anti-restriction. Curr Opin Microbiol 2005;8(4):466–472. DOI: 10.1016/J.MIB.2005.06.003.
94. Roberts RJ, Belfort M, Bestor T, et al. A nomenclature for restriction enzymes, DNA methyltransferases, homing endonucleases and their genes. Nucleic Acids Res 2003;31(7):1805–1812. DOI: 10.1093/NAR/GKG274.
95. Samson JE, Magadán AH, Sabri M, et al. Revenge of the phages: Defeating bacterial defences. Nat Rev Microbiol 2013;11(10):675–687. DOI: 10.1038/NRMICRO3096.
96. Raleigh EA, Wilson G. Escherichia coli K-12 restricts DNA containing 5-methylcytosine. Proceedings of the National Academy of Sciences. 1986;83(23):9070–9074. DOI: 10.1073/PNAS.83.23.9070.
97. Stewart FJ, Panne D, Bickle TA, et al. Methyl-specific DNA binding by McrBC, a modification-dependent restriction enzyme. J Mol Biol 2000;298(4):611–622. DOI: 10.1006/JMBI.2000.3697.
98. Bair CL, Black LW. A Type IV modification dependent restriction nuclease that targets glucosylated hydroxymethyl cytosine modified DNAs. J Mol Biol 2007;366(3):768–778. DOI: 10.1016/J.JMB.2006.11.051.
99. Hille F, Richter H, Wong SP, et al. The biology of CRISPR-Cas: Backward and forward. Cell 2018;172(6):1239–1259. DOI: 10.1016/J.CELL.2017.11.032.
100. Makarova KS, Wolf YI, Alkhnbashi OS, et al. An updated evolutionary classification of CRISPR–Cas systems. Nature Rev Microbiol 2015;13(11):722–736. DOI: 10.1038/nrmicro3569.
101. Barrangou R, Fremaux C, Deveau H, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science (1979) 2007;315(5819):1709–1712. DOI: 10.1126/science.1138140.
102. Marraffini LA, Sontheimer EJ. CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA. Science (1979) 2008;322(5909):1843–1845. DOI: 10.1126/SCIENCE.1165771/SUPPL_FILE/MARRAFFINI-SOM.PDF.
103. Jinek M, Chylinski K, Fonfara I, et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science (1979) 2012;337(6096):816–821. DOI: 10.1126/SCIENCE.1225829/SUPPL_FILE/JINEK.SM.PDF.
104. Sashital DG, Jinek M, Doudna JA. An RNA-induced conformational change required for CRISPR RNA cleavage by the endoribonuclease Cse3. Nat Struct Mol Biol 2011;18(6):680–687. DOI: 10.1038/nsmb.2043.
105. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. Cell 2015;163(3):759–771. DOI: 10.1016/J.CELL.2015.09.038.
106. Semenova E, Jore MM, Datsenko KA, et al. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. Proc Natl Acad Sci USA 2011;108(25):10098–10103. DOI: 10.1073/PNAS.1104144108/SUPPL_FILE/PNAS.1104144108_SI.PDF.
107. Deveau H, Barrangou R, Garneau JE, et al. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J Bacteriol 2008;190(4):1390–1400. DOI: 10.1128/JB.01412-07.
108. Hegge JW, Swarts DC, Van Der Oost J. Prokaryotic argonaute proteins: novel genome-editing tools? Nat Rev Microbiol 2018;16(1):5–11. DOI: 10.1038/NRMICRO.2017.73.
109. Makarova KS, Wolf YI, van der Oost J, et al. Prokaryotic homologs of argonaute proteins are predicted to function as key components of a novel system of defense against mobile genetic elements. Biol Direct 2009;4:29. DOI: 10.1186/1745-6150-4-29.
110. Swarts DC, Szczepaniak M, Sheng G, et al. Autonomous generation and loading of DNA guides by bacterial argonaute. Mol Cell 2017;65(6):985–998.e6. DOI: 10.1016/J.MOLCEL.2017.01.033.
111. Zander A, Willkomm S, Ofer S, et al. Guide-independent DNA cleavage by archaeal argonaute from Methanocaldococcus jannaschii. Nat Microbiol 2017;2. DOI: 10.1038/NMICROBIOL.2017.34.
112. Snyder L. Phage-exclusion enzymes: A bonanza of biochemical and cell biology reagents? Mol Microbiol 1995;15(3):415–420. DOI: 10.1111/J.1365-2958.1995.TB02255.X.
113. Harms A, Brodersen DE, Mitarai N, et al. Toxins, targets, and triggers: An overview of toxin-antitoxin biology. Mol Cell 2018;70(5):768–784. DOI: 10.1016/J.MOLCEL.2018.01.003.
114. Millman A, Bernheim A, Stokar-Avihail A, et al. Bacterial retrons function in anti-phage defense. Cell 2020;183(6):1551–1561.e12. DOI: 10.1016/J.CELL.2020.09.065.
115. Egido JE, Costa AR, Aparicio-Maldonado C, et al. Mechanisms and clinical importance of bacteriophage resistance. FEMS Microbiol Rev 2022;46(1):fuab048. DOI: 10.1093/FEMSRE/FUAB048.
116. Ka D, Oh H, Park E, et al. Structural and functional evidence of bacterial antiphage protection by Thoeris defense system via NAD+ degradation. Nat Commun 2020;11(1):2816. DOI: 10.1038/S41467-020-16703-W.
117. Penadés JR, Christie GE. The phage-inducible chromosomal islands: A family of highly evolved molecular parasites. Annu Rev Virol 2015;2:181–201. DOI: 10.1146/annurev-virology-031413-085446.
118. Tormo-Más MÁ, Mir I, Shrestha A, et al. Moonlighting bacteriophage proteins derepress staphylococcal pathogenicity islands. Nature 2010;465(7299):779–782. DOI: 10.1038/nature09065.
119. Novick RP, Christie GE, Penadés JR. The phage-related chromosomal islands of gram-positive bacteria. Nat Rev Microbiol 2010;8(8):541–551. DOI: 10.1038/NRMICRO2393.
120. Ram G, Chen J, Ross HF, et al. Precisely modulated pathogenicity island interference with late phage gene transcription. Proc Natl Acad Sci U S A 2014;111(40):14536–14541. DOI: 10.1073/PNAS. 1406749111.
121. Ruzin A, Lindsay J, Novick RP. Molecular genetics of SaPI1 – A mobile pathogenicity island in Staphylococcus aureus. Mol Microbiol 2001;41(2):365–377. DOI: 10.1046/J.1365-2958.2001.02488.X.
122. Ram G, Chen J, Kumar K, et al. Staphylococcal pathogenicity island interference with helper phage reproduction is a paradigm of molecular parasitism. Proc Natl Acad Sci U S A 2012;109(40): 16300–16305. DOI: 10.1073/PNAS.1204615109.
123. Rousset F, Dowding J, Bernheim A, et al. Prophage-encoded hotspots of bacterial immune systems. bioRxiv. Published online January 22, 2021:2021.01.21.427644. DOI: 10.1101/2021.01.21.427644.
124. Goldfarb T, Sberro H, Weinstock E, et al. BREX is a novel phage resistance system widespread in microbial genomes. EMBO J 2015;34(2):169–183. DOI: 10.15252/EMBJ.201489455.
125. Gordeeva J, Morozova N, Sierro N, et al. BREX system of Escherichia coli distinguishes self from non-self by methylation of a specific DNA site. Nucleic Acids Res 2019;47(1):253–265. DOI: 10.1093/NAR/GKY1125.
126. Ofir G, Melamed S, Sberro H, et al. DISARM is a widespread bacterial defence system with broad anti-phage activities. Nat Microbiol 2018;3(1):90–98. DOI: 10.1038/S41564-017-0051-0.
127. Guo H, Arambula D, Ghosh P, et al. Diversity-Generating Retroelements in Phage and Bacterial Genomes. Microbiol Spectr 2014;2(6). DOI: 10.1128/MICROBIOLSPEC.MDNA3-0029-2014.
128. Paul BG, Burstein D, Castelle CJ, et al. Retroelement guided protein diversification abounds in vast lineages of bacteria and archaea. Nat Microbiol 2017;2:17045. DOI: 10.1038/NMICROBIOL.2017.45.
129. Bertozzi Silva J, Storms Z, Sauvageau D. Host receptors for bacteriophage adsorption. FEMS Microbiol Lett 2016; 363(4):fnw002. DOI: 10.1093/FEMSLE/FNW002.
130. Latka A, Maciejewska B, Majkowska-Skrobek G, et al. Bacteriophage-encoded virion-associated enzymes to overcome the carbohydrate barriers during the infection process. Appl Microbiol Biotechnol 2017;101(8):3103–3119. DOI: 10.1007/S00253-017-8224-6.
131. Rusinov IS, Ershova AS, Karyagina AS, et al. Avoidance of recognition sites of restriction-modification systems is a widespread but

not universal anti-restriction strategy of prokaryotic viruses. BMC Genomics 2018;19(1):1–11. DOI: 10.1186/S12864-018-5324-3/FIGURES/4.
132. Rocha EPC, Danchin A, Viari A. Evolutionary role of restriction/modification systems as revealed by comparative genome analysis. Genome Res 2001;11(6):946–958. DOI: 10.1101/GR.GR-1531RR.
133. Labrie SJ, Samson JE, Moineau S. Bacteriophage resistance mechanisms. Nat Rev Microbiol 2010;8(5):317–327. DOI: 10.1038/NRMICRO2315.
134. Golovenko D, Manakova E, Tamulaitiene G, et al. Structural mechanisms for the 5'-CCWGG sequence recognition by the N- and C-terminal domains of EcoRII. Nucleic Acids Res 2009;37(19):6613. DOI: 10.1093/NAR/GKP699.
135. Iida S, Streiff MB, Bickle TA, et al. Two DNA antirestriction systems of bacteriophage P1, darA, and darB: Characterization of darA-phages. Virology 1987;157(1):156–166. DOI: 10.1016/0042-6822(87)90324-2.
136. Zavilgelsky GB, Kotova VY. Antirestriction activity of the monomeric and dimeric forms of T7 Ocr. Mol Biol 2014;48(1):150–157. DOI: 10.1134/S0026893313060174.
137. Hill C, Miller LA, Klaenhammer TR. In vivo genetic exchange of a functional domain from a type II A methylase between lactococcal plasmid pTR2030 and a virulent bacteriophage. J Bacteriol 1991;173(14):4363–4370. DOI: 10.1128/JB.173.14.4363-4370.1991.
138. Malone LM, Birkholz N, Fineran PC. Conquering CRISPR: How phages overcome bacterial adaptive immunity. Curr Opin Biotechnol 2021;68:30–36. DOI: 10.1016/J.COPBIO.2020.09.008.
139. Tao P, Wu X, Rao V. Unexpected evolutionary benefit to phages imparted by bacterial CRISPR-Cas9. Sci Adv 2018;4(2). DOI: 10.1126/SCIADV.AAR4134.
140. Stanley SY, Maxwell KL. Phage-Encoded Anti-CRISPR Defenses. Annu Rev Genet 2018;52:445–464. DOI: 10.1146/ANNUREV-GENET-120417-031321.
141. Blower TR, Evans TJ, Przybilski R, et al. Viral evasion of a bacterial suicide system by RNA-based molecular mimicry enables infectious altruism. PLoS Genet 2012;8(10):e1003023. DOI: 10.1371/JOURNAL.PGEN.1003023.
142. Otsuka Y, Yonesaki T. Dmd of bacteriophage T4 functions as an antitoxin against Escherichia coli LsoA and RnlA toxins. Mol Microbiol 2012;83(4):669–681. DOI: 10.1111/J.1365-2958.2012.07975.X.
143. Samson JE, Bélanger M, Moineau S. Effect of the abortive infection mechanism and Type III toxin/antitoxin system AbiQ on the lytic cycle of Lactococcus lactis phages. J Bacteriol 2013;195(17):3947. DOI: 10.1128/JB.00296-13.
144. Bingham R, Ekunwe SIN, Falk S, et al. The major head protein of bacteriophage T4 binds specifically to elongation factor Tu. J Biol Chem 2000;275(30):23219–23226. DOI: 10.1074/JBC.M0025 46200.
145. Isaev A, Drobiazko A, Sierro N, et al. Phage T7 DNA mimic protein Ocr is a potent inhibitor of BREX defence. Nucleic Acids Res 2020;48(10):5397–5406. DOI: 10.1093/NAR/GKAA290.
146. Townsend EM, Kelly L, Muscatt G, et al. The human gut phageome: origins and roles in the human gut microbiome. Front Cell Infect Microbiol 2021;11:643214. DOI: 10.3389/FCIMB.2021.643214/ BIBTEX.
147. de Abreu VAC, Perdigão J, Almeida S. Metagenomic approaches to analyze antimicrobial resistance: an overview. Front Genet 2021;11:575592. DOI: 10.3389/FGENE.2020.575592/BIBTEX.
148. Sukhum KV, Diorio-Toth L, Dantas G. Genomic and metagenomic approaches for predictive surveillance of emerging pathogens and antibiotic resistance. Clin Pharmacol Ther 2019;106(3):512–524. DOI: 10.1002/CPT.1535.
149. Churko JM, Mantalas GL, Snyder MP, et al. Overview of high throughput sequencing technologies to elucidate molecular pathways in cardiovascular diseases. Circ Res 2013;112(12):1613–1623. DOI: 10.1161/CIRCRESAHA.113.300939.
150. Pires DP, Melo LDR, Vilas Boas D, et al. Phage therapy as an alternative or complementary strategy to prevent and control biofilm-related infections. Curr Opin Microbiol 2017;39:48–56. DOI: 10.1016/J.MIB.2017.09.004.
151. Schuch R, Nelson D, Fischetti VA. A bacteriolytic agent that detects and kills Bacillus anthracis. Nature 2002;418(6900):884–889. DOI: 10.1038/nature01026.
152. Mulani MS, Kamble EE, Kumkar SN, et al. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front Microbiol 2019;10:403107. DOI: 10.3389/FMICB.2019.00539/BIBTEX.
153. Torres-Barceló C, Hochberg ME. Evolutionary rationale for phages as complements of antibiotics. Trends Microbiol 2016;24(4):249–256. DOI: 10.1016/j.tim.2015.12.011.
154. Motlagh AM, Bhattacharjee AS, Goel R. Biofilm control with natural and genetically-modified phages. World J Microbiol Biotechnol 2016;32(4):1–10. DOI: 10.1007/S11274-016-2009-4.
155. Abedon ST. Phage-Antibiotic combination treatments: Antagonistic impacts of antibiotics on the pharmacodynamics of phage therapy? Antibiotics 2019;8(4):182. DOI: 10.3390/ANTIBIOTICS8040182.
156. Liu CG, Green SI, Min L, et al. Phage-antibiotic synergy is driven by a unique combination of antibacterial mechanism of action and stoichiometry. mBio 2020;11(4):1–19. DOI: 10.1128/MBIO.01462-20/SUPPL_FILE/MBIO.01462-20-SF005.TIF.
157. Principi N, Silvestri E, Esposito S. Advantages and limitations of bacteriophages for the treatment of bacterial infections. Front Pharmacol 2019;10(MAY):457104. DOI: 10.3389/FPHAR.2019.00513/BIBTEX.
158. Blanco-Picazo P, Fernández-Orth D, Brown-Jaque M, et al. Unravelling the consequences of the bacteriophages in human samples. Sci Rep 2020;10(1):1–10. DOI: 10.1038/s41598-020-63432-7.
159. Qi H, Lu H, Qiu HJ, et al. Phagemid vectors for phage display: Properties, characteristics and construction. J Mol Biol 2012;417(3):129–143. DOI: 10.1016/J.JMB.2012.01.038.
160. Ish-Horowicz D, Burke JF. Rapid and efficient cosmid cloning. Nucleic Acids Res 1981;9(13):2989. DOI: 10.1093/NAR/9.13.2989.
161. Verbeken G, Pirnay JP, Lavigne R, et al. Call for a dedicated European legal framework for bacteriophage therapy. Arch Immunol Ther Exp (Warsz) 2014;62(2):117–129. DOI: 10.1007/S00005-014-0269-Y/FIGURES/2.
162. Yilmaz C, Colak M, Yilmaz BC, et al. Bacteriophage therapy in implant-related infections: An experimental study. J Bone Joint Surg Am 2013;95(2):117–125. DOI: 10.2106/JBJS.K.01135.
163. Kortright KE, Chan BK, Koff JL, et al. Phage therapy: A renewed approach to combat antibiotic-resistant bacteria. Cell Host Microbe 2019;25(2):219–232. DOI: 10.1016/J.CHOM.2019.01.014.
164. Sybesma W, Rohde C, Bardy P, et al. Silk route to the acceptance and re-implementation of bacteriophage therapy-Part II. Antibiotics (Basel) 2018;7(2). DOI: 10.3390/ANTIBIOTICS7020035.
165. Pirnay JP, Verbeken G, Ceyssens PJ, et al. The magistral phage. Viruses 2018;10(2):64. DOI: 10.3390/V10020064.
166. Brown R, Lengeling A, Wang B. Phage engineering: How advances in molecular biology and synthetic biology are being utilized to enhance the therapeutic potential of bacteriophages. Quant Biol 2017;5(1):42–54. DOI: 10.1007/S40484-017-0094-5.
167. Kutter E, De Vos D, Gvasalia G, et al. Phage therapy in clinical practice: treatment of human infections. Curr Pharm Biotechnol 2010;11(1):69–86. DOI: 10.2174/138920110790725401.

ORIGINAL RESEARCH

Evaluating Practice Consistency: Complying with the Directive to Obtain Umbilical Cord Arterial and Venous Blood Gasses, and Hemoglobin Values, at High-risk Deliveries

Sarah M Tweddell[1], Timothy M Bahr[2], Robin K Ohls[1], Robert D Christensen[1,2]

Received on: 13 September 2023; *Accepted on*: 10 October 2023; *Published on*: 05 January 2024

Abstract

Introduction: Using Intermountain Health multihospital data, we quantified compliance with the American College of Obstetricians and Gynecologists directive to obtain umbilical cord arterial and venous blood gasses at high-risk deliveries. We also quantified compliance with our local directive to obtain hemoglobin with the cord gasses as an early screen for anemia.

Methods: Retrospective 24-month analysis of Intermountain Health deliveries.

Results: One-thousand-fifty births had "placental abruption" mentioned in the peripartum notes. These constituted our high-risk delivery study cohort. Of these, 726 (69%) had both a cord arterial and venous sample reported; 707 (67%) also had hemoglobin reported. In 86 (8%) only one (arterial or venous) was reported, and 293 (23%) had neither gasses nor hemoglobin. One-hundred-seven of the 726 had acidosis (cord arterial pH <7.13) and 619 did not (pH ≥7.13). Among those with acidosis, 82 had abruption confirmed after birth; in 25 abruption was not confirmed. Paired umbilical arterial vs venous hemoglobin levels revealed the novel observation that umbilical venous hemoglobin is slightly lower than arterial ($p < 0.0001$), perhaps due to maternal-to-fetal acellular fluid transfer. Among the 707 that had cord hemoglobin reported, fetal/neonatal anemia was diagnosed in 83 (12%) (defined as hemoglobin below the fifth percentile lower reference interval for gestational age).

Conclusions: We see an opportunity to improve compliance with the directives to obtain cord arterial and venous blood gas and hemoglobin at high-risk births. Doing so will allow rapid evaluation of about 30% more high-risk infants for the presence of acidosis and anemia at birth.

Keywords: Abruption, Acidosis, Artery vs vein, Hemoglobin, High-risk delivery, Umbilical.

Newborn (2023): 10.5005/jp-journals-11002-0075

Introduction

In 2006, the American College of Obstetricians and Gynecologists published a Committee Opinion recommending that umbilical cord arterial and venous blood gas determinations should both be obtained at high-risk deliveries.[1] In addition to that recommendation, at Intermountain Health we request that a hemoglobin measurement be obtained with every umbilical cord gas, as a means of rapidly identifying anemia at birth, among these high-risk neonates.[2–4]

We were uncertain how frequently both an umbilical arterial and a venous blood gas, each with a hemoglobin level, are successfully being drawn and reported at our high-risk deliveries. In actual practice, the implementation of recommendations is virtually always incomplete, because barriers to full implementation exist. Steps toward more complete implementation of practice guidelines include quantifying compliance, identifying the barriers to full compliance, and working specifically to diminish or eliminate those barriers.

To assess our degree of compliance with the umbilical cord blood recommendations at high-risk births, and to begin the process of assessing implementation barriers, we performed a retrospective analysis in our multihospital system during the past 2 years. To reduce confounding variables and provide more uniformity and focus, we analyzed all deliveries where placental abruption was listed in the peripartum medical record.

Methods

Our study was a retrospective records analysis of neonates in the Legacy Intermountain Health databases. Intermountain Health is a not-for-profit organization that owns and manages hospitals in the Intermountain West of the USA. The Institutional Review Board (IRB) of Intermountain Health reviewed this proposal and approved it as exempt from the need for individual informed consent The diagnosis of placental abruption was initially screened from electronic data marts; Case Mix (the billing, coding, and financial data mart used by Intermountain Health), the extended

[1]Division of Neonatology, Department of Pediatrics, University of Utah, Salt Lake City, Utah, United States of America

[2]Division of Neonatology, Department of Pediatrics, University of Utah; Obstetric and Neonatal Operations, Intermountain Health, Salt Lake City, Utah, United States of America

Corresponding Author: Sarah Marie Tweddell, Division of Neonatology, Department of Pediatrics, University of Utah, Salt Lake City, Utah, United States of America, Phone: +1 8015817052, e-mail: Sarah.tweddell@hsc.utah.edu

How to cite this article: Tweddell SM, Bahr TM, Ohls RK, *et al*. Evaluating Practice Consistency: Complying with the Directive to Obtain Umbilical Cord Arterial and Venous Blood Gasses, and Hemoglobin Values, at High-risk Deliveries. Newborn 2023;2(4):310–313.

Source of support: Nil

Conflict of interest: Dr Robin Kjerstin Ohls and Dr Robert Dennis Christensen 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: Umbilical cord arterial and venous blood gas determinations and hemoglobin levels from $n = 1,050$ births where placental abruption was suspected before birth. Success in obtaining these laboratory tests is compared between the group where abruption was not confirmed at birth ($n = 372$) vs was confirmed at birth ($n = 678$)

Umbilical cord blood gas and Hgb measurements (n and % of total)	*Neonates where abruption was suspected, but not confirmed at birth (n = 372)*	*Neonates born after confirmed placental abruption (n = 678)*	*p-value*
Both arterial and venous cord gas	226 (60.8%)	500 (73.7%)	<0.001
Arterial gas only	13 (3.5%)	19 (2.8%)	
Venous gas only	12 (3.2%)	42 (6.2%)	
Neither	121 (32.5%)	172 (17.3%)	
Both arterial and venous Hgb	222 (59.6%)	485 (71.5%)	<0.001
Arterial Hgb only	8 (2.2%)	19 (2.8%)	
Venous Hgb only	10 (2.7%)	43 (6.3%)	
Neither	132 (35.5%)	131 (19.3%)	

Hgb, blood hemoglobin concentration

Vermont-Oxford database (EVOX), Storkbytes (the labor and delivery database), Fetal Link (a replacement for Storkbytes), provider problem lists, and International Classification of Diseases, Tenth Revision (ICD-10) coding.

The electronic medical record of every delivery that was identified by our screening ascertainment methods was individually reviewed by a member of the research team, not relying on coded information or on data tables. We did this to verify whether a diagnosis of placental abruption was confirmed after birth, and to evaluate each arterial and venous umbilical cord gas pair according to the criteria of Pomerance.[5] We judged the arterial and venous samples were mislabeled (switched) if the sample labeled "arterial" had a higher pH, a lower PCO_2, and a higher PO_2 than did the paired sample labeled "venous."

Neonates were included as having been born after a placental abruption if the obstetrician documented abruption in their procedure note, or if the pathologist confirmed abruption in their report. We attempted to characterize the size of each confirmed abruption using whatever descriptive metric we found in the medical record.

The dataset was collected and managed using an Intermountain Healthcare Research Electronic Data Capture (REDCap) electronic data capture tool; REDCap is a secure cloud-based application designed to support data capture and provide an intuitive interface for validated data entry, audit trails for tracking data manipulation and export procedures, automated export procedures for seamless data downloads to common statistical packages, and procedures for importing data from external sources. Summary statistics (means, counts, and proportions) were the primary quantitative tools used for analysis. Differences in continuous variables by group were assessed using one-way analysis of variance (ANOVA). Differences in categorical variables were assessed using either Chi-square tests or Fisher's exact test. Data management and statistical analysis were done in the R language and environment for statistical computing (R Foundation).

Results

Between 1 July 2020 and 30 June 2022, a total of 55,111 live births were recorded in our healthcare system. One-thousand-fifty of these (2%) had placental abruption mentioned in the peripartum medical record and identified by our initial ascertainment screen. Of the 1,050 live births, 678 (65%) had placental abruption subsequently confirmed at delivery, by the obstetrician or the pathologist, while in 372 (35%) an abruption was not found.

Of the 1,050 high-risk births that constituted our study cohort, both umbilical cord arterial and venous samples were reported from 726 (69%); 706 of these (67%) also had hemoglobin reported. In 86 (8%), only one sample (cord arterial or venous) was obtained, and in 293 (23%) no cord gas or hemoglobin value was reported. We found 25 paired samples that we judged to have had "switched labels," according to the criteria of Pomerance.[5] We recorded the corrected values accordingly. We were unable to determine what proportion of the umbilical cord blood tests were drawn from a double-clamped segment of the umbilical cord vs drawn from a single-clamped cord that remained attached to the placenta.[6] We were also unable to determine the time intervals between birth and drawing the sample, and between drawing the sample and running the test, including which of the specimens were transported on ice.[7]

As shown in Table 1, those where a placental abruption was confirmed were more likely to have both an arterial and a venous blood gas reported ($p < 0.001$). Those with a confirmed abruption were far less likely to have neither reported. The same occurred with cord blood hemoglobin reporting; namely, those with a confirmed abruption were more likely to have both arterial and venous hemoglobin values reported ($p < 0.001$) and were far less likely to have neither reported.

Of the 726 high-risk births where both an arterial and a venous cord blood gas value was reported, 107 had acidosis at birth, defined as a cord arterial pH <7.13 (using the definition of Johnson and Richards,[8] Table 2A) Of the 107 with acidosis, 82 had a placental abruption confirmed after birth and 25 had no evidence of an abruption. No differences in blood gasses were apparent between the group of acidotic neonates who had an abruption vs those who did not. Thus, among those with acidosis at birth, we were unable to distinguish cases of confirmed abruption based on blood gas values.

Six-hundred-nineteen of the 726 high-risk births did not have acidosis at birth (cord arterial pH ≥7.13). Four-hundred-eighteen of these had placental abruption confirmed after birth and 201 had no evidence of an abruption (Table 2B). Those with confirmed abruption were more likely to have a small difference (<0.15 pH units) between their venous and arterial pHs (suggesting poor placental function in correcting fetal acidosis, that is, their venous and arterial pHs were nearly the same)[5] (92% with abruption had this small difference vs 86% in those who did not have abruption,

Tables 2A and B: Umbilical cord venous and arterial pHs in 726 high-risk births. Table 2A includes only those who had acidosis (defined as a cord arterial pH <7.13).[8] Table 2B includes only those who did not have acidosis (defined as a cord arterial pH ≥7.13)[8]

(A) Acidosis (cord arterial pH <7.13) was present at 107 births

Venous and arterial pH	*Neonates where abruption was suspected by screening but was not confirmed at birth (n = 25*)*	*Neonates born after confirmed placental abruption (n = 82*)*	*p-value*
Difference (venous minus arterial, mean ± SD) in pH units	0.12 ± 0.11	0.11 ± 0.08	0.846
Percent with a difference of <0.15 pH units	17 (68%)	58 (71%)	0.991
Percent with a difference of ≥0.15 pH units	8 (32%)	24 (29%)	

*These analyses only include neonates for whom both a venous and arterial pH was available. SD, standard deviation

(B) Acidosis was not present (cord arterial pH ≥7.13) at 619 births

Venous and arterial pH	*Neonates where abruption was suspected by screening but was not confirmed at birth (n = 201*)*	*Neonates born after confirmed placental abruption (n = 418*)*	*p-value*
Difference (venous minus arterial, mean ± SD) in pH units	0.08 ± 0.05	0.07 ± 0.06	0.033
Percent with a difference of <0.15 pH units	173 (86%)	383 (92%)	0.046
Percent with a difference of ≥0.15 pH units	28 (14%)	35 (8%)	

*These analyses only include neonates for whom both a venous and arterial pH was available

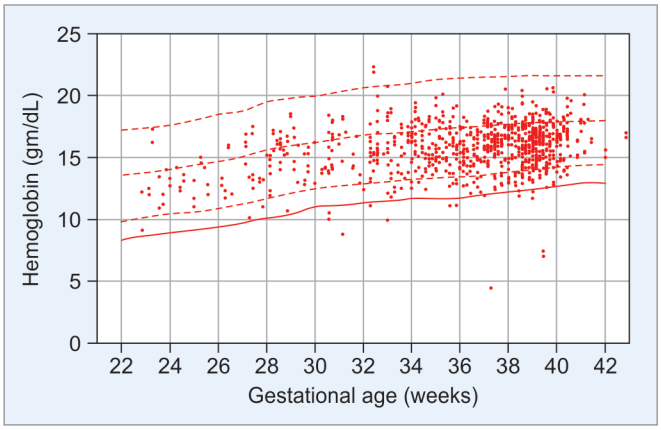


Fig. 1: Neonates were born when "placental abruption" was recorded in the perinatal medical record and had both a venous and an arterial umbilical cord blood gas, and hemoglobin values, reported. The lower dashed line indicates the fifth percentile lower reference interval (below which anemia is diagnosed) and the dark line indicates the first percentile lower reference interval (below which severe anemia is diagnosed).[3,9]

where presumably the placenta was functioning somewhat better ($p = 0.046$). This very small difference between venous and arterial pH was more common in those who did not have acidosis (Table 2B, 92%) than those who did have acidosis (Table 2A, 71%, $p < 0.001$). In 275 of the 500 confirmed abruption cases (55%) we found no indication of the abruption size, moreover the recorded abruption sizes were inconsistent and vague, making stratification of pH values according to abruption size impossible.[10]

Including a hemoglobin level with the umbilical cord blood gas proved to be a meaningful addition. As shown in Figure 1, among the 707 that had a cord hemoglobin reported, fetal/neonatal anemia was diagnosed in 83 (12%) (defined as a hemoglobin below the fifth percentile lower reference interval for gestational age), and severe anemia (defined as below the first percentile) was diagnosed in 16 (2%).[3] Of those with a confirmed abruption, 9% (63/678) had anemia at birth. Of those who did not have an abruption, 6% had anemia at birth ($p = 0.049$). In paired analyses, the venous hemoglobin level was lower (0.2 ± 0.8 gm/dL; mean ± IQR) than the arterial hemoglobin level ($p < 0.001$). The magnitude of arterial/venous hemoglobin difference did not correlate with the presence or absence of abruption or with the likelihood or severity of fetal/neonatal anemia.

Discussion

The American College of Obstetricians and Gynecologists and the American Academy of Pediatrics both recommend performing umbilical artery and venous blood gas determinations at deliveries where a fetal metabolic abnormality is suspected.[1,7] High-risk deliveries include conditions that are associated with fetal/neonatal anemia; thus, we recommend obtaining a hemoglobin value along with each arterial and venous cord blood gas as an early screen for anemia.[2,3,9] In the present study, we determined how often our delivery personnel successfully complied with these directives. We found that in 1,050 high-risk cases, blood gas was obtained from both umbilical vessels in 69% of births, and hemoglobin was obtained with the blood gas in 67%. We interpret these percentages as unsatisfactory because we are missing these important test results in about 30% of our high-risk deliveries, thus we are delaying a possible diagnosis of acidosis, as well as anemia, waiting on umbilical line placement and drawing of blood gasses and complete blood count (CBC) results.

We previously described that severe anemia at birth (hemoglobin measured within the first 6 hours after birth below the first percentile lower reference interval) was recognized and documented by caregivers in only 45% of those cases where severe anemia at birth actually occurred.[3] Obtaining a hemoglobin measurement along with umbilical cord gasses can identify anemia at birth. In our present analysis, we see much room for

improvement. In a large hospital system compliance with practice guidelines can be poor due to implementation barriers. At this point we are uncertain what these barriers are. However, surely skill in drawing these samples varies with training and experience.

In our present study, neonates who had acidosis at birth (arterial pH <7.13) did not have features on their arterial and venous blood gas that would differentiate those who had a placental abruption from those who did not have abruption. However, in neonates who did not have severe acidosis at birth (pH ≥7.13), the venoarterial pH difference was smaller in the group with confirmed placental abruption than in those who did not have abruption; namely, with abruption the arterial and venous pHs were nearly the same, indicating the abrupted placenta was failing to correct fetal acidosis. This phenomenon was originally reported by Johnson and Richards in 1997.[8] We also found that the umbilical cord venous hemoglobin was slightly lower than the umbilical cord arterial hemoglobin. We speculate that since maternal extracellular water is the source of fetal water, trace amounts of water must be transferred from mother to fetus through the placenta into the umbilical vein; thus, the hemoglobin value in the umbilical vein is typically very slightly lower (diluted) compared with that in the umbilical arteries.

We recognize limitations in our study. First, this was a retrospective analysis, relying on manual chart reviews that were only as accurate as was the reporting of those doing the patient charting. Also, we relied on the obstetrician or pathologist's report to document the abruption, but we know that estimation of the abruption size can be subjective. Another limitation includes a lack of accuracy in drawing and labeling cord blood samples. We found 25 paired samples where we judged the arterial and venous labels must have been inadvertently switched.[5] Pomerance notes several other potential errors in cord blood collection. For example, the two samples can be drawn by unknowingly sticking to the same vessel twice. This source of error is difficult to estimate or eliminate. A rare error is due to mixed venous and arterial blood, by sampling through a needle that traverses the artery and then slips into the vein next to it. We have no way of knowing how common this was in our dataset. Finally, we did not have access to cord blood after every high-risk delivery. We do not know why cord blood was not collected at all 1,050 deliveries, and whether their exclusion results in biases in our analysis. Moreover, we are uncertain what barriers exist to full implementation of the directive to obtain both arterial and venous cord gasses and hemoglobin at high-risk deliveries.[9]

In conclusion, we collected arterial and venous cord blood gasses, and hemoglobin, as our guidelines request, from about 70% of a high-risk delivery cohort. Thus, we see an opportunity to improve. Improvement will allow us to rapidly identify additional neonates with acidosis and also those with anemia, thereby generating timely, relevant, and sometimes critical information. We hope now that we have identified this opportunity, we can improve education and training of those charged with drawing these samples, thereby making this data rapidly available to another 30% of our high-risk deliveries.

Orcid

Timothy M Bahr https://orcid.org/0000-0002-7946-0290
Robert D Christensen https://orcid.org/0000-0001-5872-582X

References

1. ACOG Committee on Obstetric Practice. ACPG Committee Opinion No. 348: Umbilical cord blood gas and acid-base analysis. Obstet Gynecol 2006;108(5):1319–1322. DOI: 10.1097/00006250-200611000-00058.
2. Carr NR, Henry E, Bahr TM, et al. Fetomaternal hemorrhage: Evidence from a multihospital healthcare system that up to 40% of severe cases are missed. Transfusion 2022;62:60–70. DOI: 10.1111/trf.16710.
3. Bahr TM, Lawrence SM, Henry E, et al. Severe anemia at birth-incidence and implications. J Pediatr 2022;248:39–45.e2. DOI: 10.1016/j.jpeds.2022.05.045.
4. Carr NR, Hulse WL, Bahr TM, et al. First report of transfusing low-titer cold-stored type O whole blood to an extremely-low-birth-weight neonate after acute blood loss. Transfusion 2022;62:1923–1926. DOI: 10.1111/trf.17034.
5. Pomerance J. Umbilical cord blood gas casebook. Interpreting umbilical cord blood gases, II. J Perinatol 1998;18(2):160–161. PMID: 9605310.
6. Simhan HV. Umbilical cord blood acid–base analysis at delivery. UpToDate 2 Feb 02 2023.
7. American College of Obstetricians and Gynecologists (ACOG) and American Academy of Pediatrics (AAP). Neonatal Encephalopathy and Neurologic Outcome, 2nd edition. Washington, DC: ACOG; 2014. Reaffirmed 2020.
8. Johnson JW, Richards DS. The etiology of fetal acidosis as determined by umbilical cord acid–base studies. Am J Obstet Gynecol 1997;177(2):274–280. DOI: 10.1016/s0002-9378(97)70187-x.
9. Christensen RD, Bahr TM, Tweddell SM, et al. An evidence-based approach to diagnosing anemia in neonates. Neoreviews 2023;24(6):e343–e355. DOI: 10.1542/neo.24-6-e343.
10. Tweddell SM, Bahr TM, Henry E, et al. Placental abruption, and neonatal anemia. J Perinatol 2023;43(6):782–866. DOI: 10.1038/s41372-023-01603-w.

PHOTO ASSAY

Image: Identifying Malposition of Umbilical Venous Catheter Using Lateral Film

Archana Bottu[1], Lakeya O'Neal[1], Shabih Manzar[1]

Received on: 10 November 2023; *Accepted on*: 11 December 2023; *Published on*: 05 January 2024

Abstract

The umbilical venous catheters (UVC) are frequently used in premature infants for intravenous access in the early postnatal period. The position of these catheters is typically verified by thoraco-abdominal radiographs, usually in the anterior-posterior view. In this case, we highlight the importance of obtaining a lateral image when a malposition is suspected.

Keywords: Congenital vascular malformation, Erroneous catheter insertion, Intrahepatic portosystemic shunts, Lateral film, Malposition, Newborn, Umbilical venous catheter.

Newborn (2023): 10.5005/jp-journals-11002-0079

Case

We inserted an umbilical venous catheter (UVC) in a premature infant born at a gestational age of 33 weeks and a birth weight of 1530 grams. The length of insertion of this UVC was estimated using a well-established method [(3 × weight in kilograms + 9)/2) + 2],[1] and a thoraco-abdominal radiograph [antero-posterior (AP), view] was obtained for confirming correct catheter placement. Interestingly, the UVC showed an unusual coiled-up track in the hepatic region (Fig. 1A). In addition to erroneous insertion, the possibility of congenital hepatic vascular abnormalities with intrahepatic portosystemic shunts came to mind.[2] Fortunately, a lateral radiograph (Fig. 1B) showed a simpler UVC track with only a single turn. Taken together, the findings in the AP view were interpreted as favoring erroneous insertion of the catheter over congenital anomalies. The line was safely removed and the subsequent clinical course of the infant was uneventful. No hepatomegaly or abnormalities in liver function/hematological laboratory panels were noted. These images reiterate the importance of obtaining lateral radiographs when UVCs show unusual tracks in AP views.

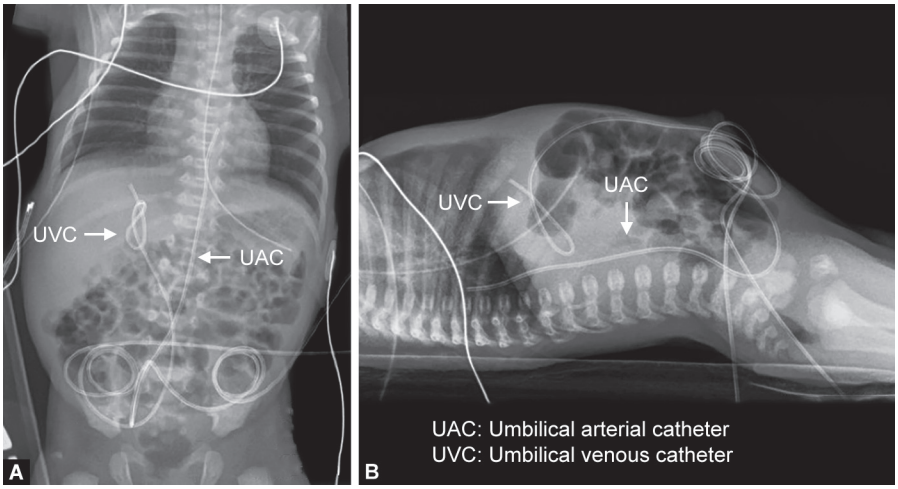
[1]Department of Neonatology/Pediatrics, Louisiana State University Health Sciences Center, Louisiana, United States of America

Corresponding Author: Shabih Manzar, Department of Neonatology/Pediatrics, Louisiana State University Health Sciences Center, Louisiana, United States of America, Phone: +1 318-675-7275, e-mail: shabih.manzar@lsuhs.edu

How to cite this article: Bottu A, O'Neal L, Manzar S. Image: Identifying Malposition of Umbilical Venous Catheter Using Lateral Film. Newborn 2023;2(4):314–315.

Source of support: Nil

Conflict of interest: None



Figs 1A and B: (A) A thoraco-abdominal radiograph (antero-posterior view) of a premature infant showed an umbilical venous catheter forming an unusual coil in the hepatic region. The track of the umbilical arterial catheter was as expected; (B) A lateral radiograph from the same infant. The track of the umbilical venous catheter showed only a single turn. The line was safely removed

Orcid

Shabih Manzar https://orcid.org/0000-0002-5621-963X

References

1. Shukla H, Ferrara A. Rapid estimation of insertional length of umbilical catheters in newborns. Am J Dis Child 1986;140(8):786–788. DOI: 10.1001/archpedi.1986.02140220068034.
2. Schmalz MJ, Radhakrishnan K. Vascular anomalies associated with hepatic shunting. World J Gastroenterol 2020;26(42):6582–6598. DOI: 10.3748/wjg.v26.i42.6582.