January-March 2026 Volume 5 Issue 1 newborn Official Journal of the Global Newborn Society eISSN: 2769-514X

newborn

January-March 2026
Volume 5
Issue 1
eISSN 2769-514X

Official Journal of the Global Newborn Society and 52 allied organizations
International Society for Marginalized Lives
Dr. Mozib Newborn Foundation
The Carlo GNS Center for Saving Lives at Birth
Vishwa Mahesh Parivaar
Autism Care Network Foundation

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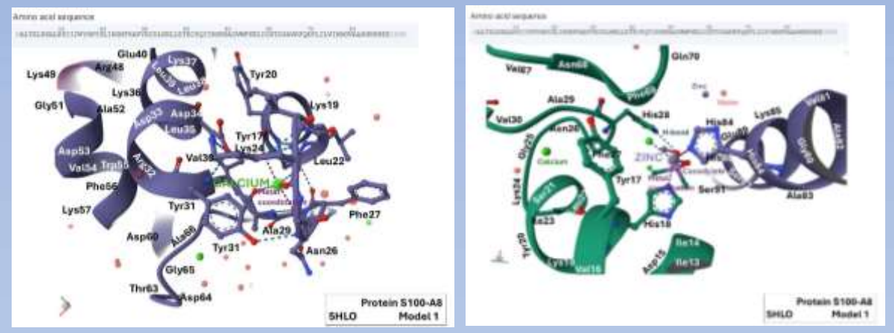
Anatolian Midwives Association
GNS Western Australia
Perinatal Society of Singapore
Pioneers - looking for sustainable ways to reduce infant mortality
Bhutan Neonatal Care Forum
Global Newborn Society Iran Chapter
National Federation of Neonatologists of Mexico
College of Neonatologists of the State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

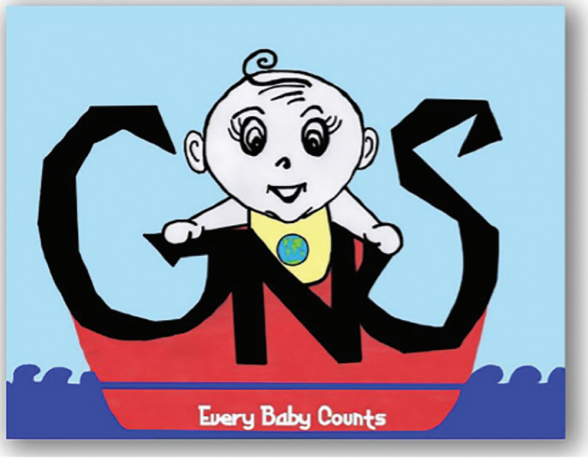


Calprotectin: An Overview
Figure shows Ca^{++} and Zn^{++} ions inside the S100A8 peptides

Other highlighted articles:
Impact of the Ukrainian Conflict on Mothers and Young Infants Who Have Had to Migrate to Poland
Meckel diverticulum

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 5, Issue 1; January–March 2026

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-5-1-iv

A Combination of Human Wisdom and Artificial Intelligence will Win the Game

There is concern in academia about how artificial intelligence (AI) will change learning, and our society.[1-3] We have a different view. As mentioned in earlier editorials, we see advancing AI not as an artificial change but as a combination of supplementary and supplemental intelligence.[4] As shown in **Figure 1**, we have highlighted that a combination of human qualities and AI together could/will be a winning strategy. Humans will bring creativity, ethical judgment, clinical insight, and contextual understanding, and electronic processing will provide speedy calculations, pattern recognition, and the ability to quickly/efficiently analyze massive datasets. This synergistic model will bring speed—machines will likely enhance, not replace human decision-making. We will no longer have the guilt about breaks for meals and personal needs, and may actually gain time to dream, think, and plan orbital jumps in our goals. Advances from organizations such as DeepMind and developments in systems like AlphaFold illustrate how human–AI collaboration can solve complex scientific problems that neither could address alone.[5-9] In the following paragraphs, we expand on the reasons for our thinking based on extensive discussions with our colleagues engaged in the development of AI algorithms:

1. **Pattern recognition and hidden structures:** AI enables the identification of complex, multidimensional patterns within large datasets that might have been difficult to detect using conventional statistical approaches.[10-12] Machine learning algorithms can recognize subtle correlations, nonlinear relationships, and hierarchical structures across thousands or millions of variables simultaneously.[13-17] Deep learning systems developed by DeepMind, such as AlphaFold, have shown how AI can uncover intricate biological patterns from massive protein sequence datasets.[18] By revealing hidden structures in data, AI can transform raw information into meaningful insights.[19,20]
2. **Scalability and high-throughput data processing:** Large-scale scientific technologies generate enormous volumes of data that exceed the capacity of manual analysis.[21,22] AI provides scalable computational frameworks capable of processing high-throughput sequencing data, continuous physiological monitoring streams, and large imaging repositories efficiently and consistently.[23-27] Automated pipelines powered by AI reduce human error, enhance reproducibility, and enable real-time analysis.[28,29] This scalability allows researchers to move beyond small datasets and engage with population-level or genome-wide investigations.[30,31]
3. **Predictive modeling and forecasting:** AI inspires data analysis by shifting focus from descriptive interpretation to predictive insight.[32,33] Through supervised learning techniques, models can be trained on historical data to forecast outcomes, classify disease states, or estimate risk probabilities.[34,35] These predictive capabilities are particularly powerful in clinical research, where AI can anticipate complications, stratify patients based on risk, and support personalized decision-making.[12,36] By modeling nonlinear and high-dimensional relationships, AI often achieves greater predictive accuracy than traditional regression-based approaches.[37,38]
4. **Unsupervised discovery and hypothesis generation:** Beyond prediction, AI supports exploratory analysis through unsupervised learning methods that identify natural groupings or latent structures without predefined labels.[39,40] Clustering algorithms, dimensionality reduction techniques, and network-based analyses reveal new subtypes, biological pathways, or evolutionary

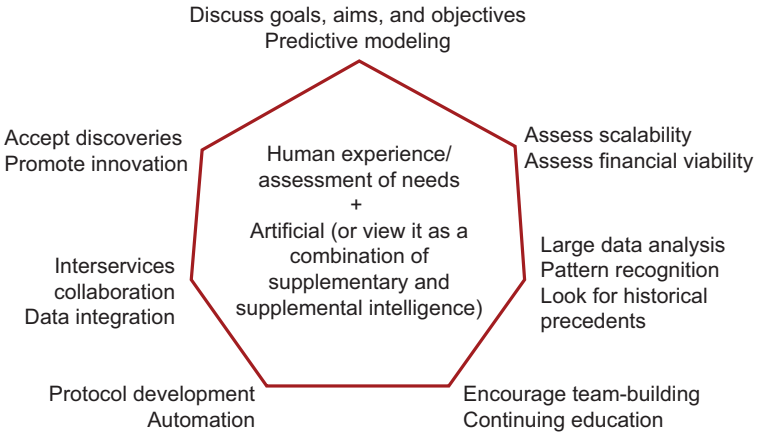


Fig. 1: We need to recognize possible advantages of combining human wisdom with artificial intelligence (AI). We have mentioned in earlier issues of the journal – is AI even the right word – should we not view these advances as a combination of supplemental and supplementary intelligence? In this heptagon, we have highlighted that a combination of human qualities and AI together would be a winning strategy. Humans will bring creativity, ethical judgment, clinical insight, and contextual understanding, and electronic processing will provide speedy calculations, pattern recognition, and the ability to quickly/efficiently analyze massive datasets. This synergistic model will bring speed - machines will likely enhance, not replace human decision-making. We will no longer have the guilt about breaks for meals and personal needs, and may actually gain time to dream, think, and plan orbital jumps in our goals. Advances from organizations such as DeepMind and developments in systems like AlphaFold illustrate how human–AI collaboration can solve complex scientific problems that neither could address alone. Ultimately, the future of innovation is likely to depend on integrating human reasoning with adaptive AI. Let float and swim in this stream – we will enjoy it

relationships.[41] This capacity for unbiased discovery encourages hypothesis generation and supports innovative research directions.[42] Rather than testing only predetermined assumptions, AI may help in formulation of new questions.[43,44]

5. **Integration of heterogeneous data sources:** Modern research frequently involves integrating diverse data types, including genomic sequences, imaging data, electronic health records, and environmental variables.[45] AI provides frameworks capable of combining these heterogeneous datasets into unified analytical models. Such integrative analysis enables the identification of cross-domain relationships that may not be evident when datasets are examined independently.[46] By synthesizing multiple layers of information, AI promotes systems-level understanding of complex biological and clinical phenomena.[47]
6. **Automation and efficiency enhancement:** AI reduces the burden of repetitive and computationally intensive tasks, including feature extraction, anomaly detection, image segmentation, and sequence alignment optimization.[36] Automation can accelerate research workflows and improve consistency across analyses.[48] As a result, researchers will likely be able to allocate more time to interpretation, critical thinking, and theoretical development.[49]
7. **Continuous learning and adaptation:** Unlike static analytical methods, AI systems can continuously learn from new incoming data and refine their models over time.[12,50] Adaptive algorithms improve performance as datasets expand, making them particularly suitable for dynamic environments such as clinical monitoring systems or evolving epidemiological studies.[51,52] This ability to update knowledge in real time can provide updated insights that will remain responsive to new evidence.

To conclude, the integration of human intelligence with AI represents a transformative and highly effective approach to large-scale data analysis.[10] While AI offers unparalleled computational capacity, scalability, and advanced pattern recognition, human expertise provides contextual understanding, ethical judgment, creativity, and critical interpretation.[53,54] Together, this can be synergistic partnership.

In each issue of this journal, our editorial team highlights the achievements of one of our partnering members. Here, we present the Instituto Nacional de Perinatología Isidro Espinosa de los Reyes, which is associated with the National Health Institutes of México (NHIM), in Mexico City.[55] It was founded in 1977 by Dr. Eduardo Jurado Garcia, who led the facility as its first Director.[56] The three main activities are identified as healthcare, education, and research. The NHIM is a renowned referral center for women and neonatal health care, with facilities for high-risk gynecology, reproductive health, maternal-fetal medicine with fetal surgery, fetal cardiology, neonatology, genetics, and oncology **(Fig. 2)**. The newborn center includes an intensive care unit with 75 beds; 16 are earmarked for specialized ventilation, surgery, neurosurgery, hypothermia, and areas for rooming-in. It is equipped for high-frequency ventilation, inhaled nitride oxide, neonatal infrared spectroscopy, incubators for magnetic resonance, equipment for hypothermia, and a specifically equipped neonatal ambulance for transport. There is an associated human milk bank. There is a well-recognized follow-up program. The facility is attached with largest neonatologist training center in Mexico, which collaborates with the Universidad Nacional Autónoma de Mexico (National Autonomous University of Mexico) and graduates about 30 new neonatologists each year.

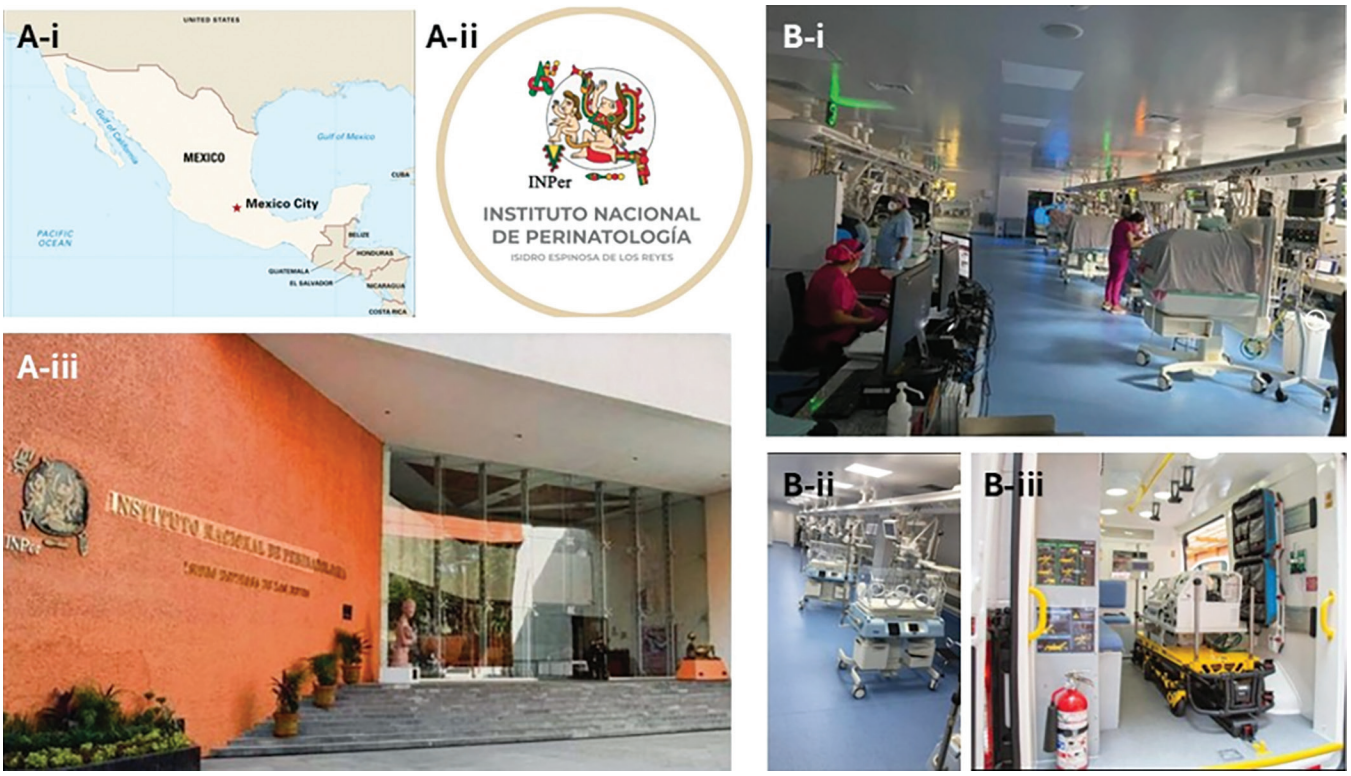


Fig. 2: Instituto Nacional de Perinatología Isidro Espinosa de los Reyes, which is associated with the National Health Institutes of México (A-i) located in Mexico City; (A-ii) The logo of the Institute, Princesa 3 Pedernal (Princess 3 Flintstone), comes from Codex Nutall found in the región of Oaxaca; (A-iii) The hospital entrance. Figures B (i–iii) show the neonatal intensive care unit, facilities, and the transport service, respectively

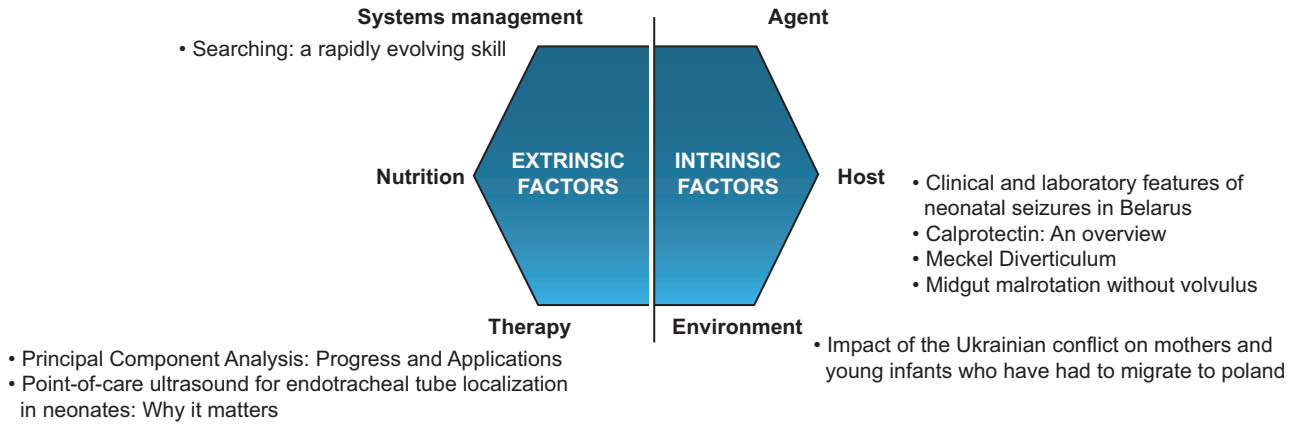


Fig. 3: Areas of focus in the *newborn*, Volume 5, Issue 1. We have expanded the traditional agent-host-environment trinodal disease model to a hexagonal system. The three additional foci represent extrinsic factors that can affect health - those originating in therapy, nutrition, and systems management are shown. This issue covers 3 nodes, with articles focused on host factors, therapy/monitoring systems, and systems management.

This journal aims to cover fetal/neonatal problems that begin during pregnancy, at the time of birth, or during the first 1,000 days after birth. As in our previous issues, we present 8 articles here **(Fig. 3)**. Pratasevich described the clinical and laboratory features of neonatal seizures in Belarus.[57] Although Belarus has now one of lowest neonatal mortality figures in the world,[58] seizures continue to be seen in infants. The authors reviewed the clinical features of 75 neonates with seizures in the Grodno region. Hypoxic-ischemic encephalopathy, perinatal infections, metabolic disorders, and intracranial hemorrhages were the leading causes. Seizures were seen most often in term infants in the early neonatal period. The authors continue to develop the cohort in a prospective fashion. As requested by the readers, we, as editors, are also introducing consistent series focused on imaging[59] and procedures.[60]

Frydrysiak-Brzozowska and her colleagues[61] have reported the impact of the war in Ukraine on pregnant mothers and their infants who have had to relocate to Poland. There has been widespread destruction, displacement, and humanitarian crises across Ukraine.[62] Over 2 million people had to flee Ukraine within 2 weeks and there have been many more border crossings.[63] In this article, the authors have focused on the social impact of population migration, with particular attention to its effects on perinatal outcomes. Thousands of Ukrainian women were pregnant when the war began and the conflict affected their care with lack of access to medical facilities and high levels of stress. Their infants also need care. The arrival of large numbers of refugees has changed Poland's social landscape.[64] However, the Polish government and society have responded to these population movements by offering all-possible protection and wide-ranging support.[65] The country deserves global recognition and gratitude.

In an important review article, Maheshwari, Mohammadabadi, and Michie have described our current understanding of the biological/clinical importance of calprotectin, a heterodimeric calcium- and zinc-binding innate immunity-related protein.[66,67] Neonates rely on innate immunity for survival and can show higher calprotectin levels than older children and adults.[68-70] Serum calprotectin levels are increased during systemic inflammation, further highlighting its important contribution to immune responses.[71] In gut disorders, fecal calprotectin is a useful noninvasive marker that can help differentiate inflammatory bowel conditions from functional gastrointestinal disorders, assess the severity of disease activity, and monitor therapeutic responses.[71] It is a valuable diagnostic and monitoring tool that can enhance decision-making while reducing reliance on invasive investigations.[72]

Khare has reviewed the embryology, clinical features, and management of Meckel diverticulum (MD).[73] These are congenital 'pouches' located on the antimesenteric border of the distal ileum, seen worldwide in about 2% of all infants.[74,75] The etiopathogenesis is not clear. There can be three possible variants of MDs: (a) a cyst with the rest of the omphalomesenteric duct (OMD) seen as a terminal fibrous band connecting with the umbilicus; (b) the whole OMD remains patent; and (c) an umbilical cyst, a fluid-filled remnant in a part of the ductal pathway.[74,76] Asymptomatic patients may be closely followed with conservative management. However, for symptomatic cases, surgical management with a diverticulectomy or a segmental ileal resection may have to be considered.[77]

Maheshwari and colleagues[78] formed a team with authors from all continents to review how Principal Component Analysis (PCA) is done/understood in the analysis of high-throughput arrays.[79] This work is important because of the existing/needed interface between clinicians and computer software experts because of differing areas of expertise. Simply put, PCA is a statistical technique used to reduce the dimensionality of large datasets while preserving as much important information as possible.[79] Gene expression data typically contain thousands of variables, something strikingly different from the clinical thought process. PCA reduces this high dimensionality into a smaller set of variables.[80] Progress in these sections can help researchers better visualize complex expression patterns, identify clusters of samples, detect outliers, and uncover underlying biological differences between health and diseased states.

Anil Rao has contributed a pictorial essay of plain and contrast-enhanced gastrointestinal images from an infant with intestinal malrotation.[59,81] Gut malrotation is a congenital condition in which the intestines become fixed in an abnormal position during fetal development and do not rotate further.[82] This condition presents most frequently during infancy with symptoms of intestinal obstruction, although some individuals may remain asymptomatic until later in childhood or adolescence. The diagnosis is typically made using imaging studies, and treatment usually involves surgical correction to prevent serious complications. The pathophysiology of malrotation is not well known, although mutations or altered expression of some genes involved in laterality and gut morphogenesis

are now being discovered.[83,84] The management of these infants involves supportive management and surgical intervention to prevent life-threatening complications such as midgut volvulus.[85,86]

Michie and Maheshwari[87] discuss on how our methods for online search for topics are changing. With the advent of artificial intelligence (AI) neural networks, the debate about the convenience and accuracy of medical or scientific information obtained from published material, search engines, and AI tools is becoming ever stronger.[88-90] A new era of questioning - 'why', 'how' and 'what if' – the Socratic approach seems to be appearing again.[91,92] We not only need careful validation of AI large language models, but there are also concerns that the ease of computation-enabled linear thinking could limit serendipity.[93-95]

Finally, we are developing a few series of procedure-focused articles to standardize the approach all over the world. Monika Kaushal from the United Arab Emirates has provided a brief summary of the sonographic confirmation of endotracheal tube (ETT) position in newborn infants.[60] In neonatal intensive care units, ETT malposition is a frequently encountered difficulty in management of critically ill infants.[96] Chest radiography still remains the gold standard for confirmation of correct positioning of the ETT, but it is limited by temporal delays, radiation exposure, and workflow inefficiencies.[97] Point-of-care ultrasound (POCUS) offers a rapid, radiation-free, and reliable alternative for confirming the position of the ETT at the bedside. This review summarizes the clinical rationale, scanning technique, available evidence, and practical strategies for using POCUS for ETT localization in neonates.

References

1. Klimova B, Pikhart M. Exploring the effects of artificial intelligence on student and academic well-being in higher education: a mini-review. Front Psychol. 2025;16:1498132. PMID: 39963679. doi: 10.3389/fpsyg.2025.1498132.
2. Al-Zahrani AM, Alasmari TM. Exploring the impact of artificial intelligence on higher education: The dynamics of ethical, social, and educational implications. Humanit Soc Sci Commun. 2024;11:912. doi: 10.1057/s41599-024-03432-4.
3. Tai MC. The impact of artificial intelligence on human society and bioethics. Tzu Chi Med J. 2020;32(4):339–343. PMID: 33163378. doi: 10.4103/tcmj.tcmj_71_20.
4. Maheshwari A, Lui K, Motta M. Are we ready to view artificial intelligence not as a threat but as supplementary and/or supplemental intelligence? Newborn (Clarksville, Md). 2025;4(1):iv-x. doi: 10.5005/newborn-4-1-iv.
5. Powles J, Hodson H. Google DeepMind and healthcare in an age of algorithms. Health Technol (Berl). 2017;7(4):351–367. PMID: 29308344. doi: 10.1007/s12553-017-0179-1.
6. Elliott LG, Simpkin AJ, Rigden DJ. ABCFold: easier running and comparison of AlphaFold 3, Boltz-1, and Chai-1. Bioinform Adv. 2025;5(1):vbaf153. PMID: 40708869. doi: 10.1093/bioadv/vbaf153.
7. Thangamalai MS, Desai D, Selvaraj C. Revolutionizing structural biology: AI-driven protein structure prediction from AlphaFold to next-generation innovations. Adv Protein Chem Struct Biol. 2025;147:1–19. PMID: 40973394. doi: 10.1016/bs.apcsb.2025.04.002.
8. Szczepski K, Jaremko L. AlphaFold and what is next: bridging functional, systems and structural biology. Expert Rev Proteomics. 2025;22(2):45–58. PMID: 39824781. doi: 10.1080/14789450.2025.2456046.
9. Wei GW. Protein structure prediction beyond AlphaFold. Nat Mach Intell. 2019;1(8):336–337. PMID: 38515561. doi: 10.1038/s42256-019-0086-4.
10. Bajwa J, Munir U, Nori A, Williams B. Artificial intelligence in healthcare: transforming the practice of medicine. Future Healthc J. 2021;8(2):e188–e194. PMID: 34286183. doi: 10.7861/fhj.2021-0095.
11. Khan SN, Danishuddin, Khan MWA, Guarnera L, Akhtar SMF. Multimodal AI in precision medicine: integrating genomics, imaging, and EHR data for clinical insights. Front Artif Intell. 2025;8:1743921. PMID: 41573270. doi: 10.3389/frai.2025.1743921.
12. Dixon D, Sattar H, Moros N, Kesireddy SR, Ahsan H, Lakkimsetti M, et al. Unveiling the influence of AI predictive analytics on patient outcomes: A comprehensive narrative review. Cureus. 2024 ;16(5):e59954. PMID: 38854327. doi: 10.7759/cureus.59954.
13. Woodman RJ, Mangoni AA. A comprehensive review of machine learning algorithms and their application in geriatric medicine: present and future. Aging Clin Exp Res. 2023;35(11):2363–2397. PMID: 37682491. doi: 10.1007/s40520-023-02552-2.
14. Sarker IH. Machine learning: algorithms, real-world applications and research directions. SN Comput Sci. 2021;2(3):160. PMID: 33778771. doi: 10.1007/s42979-021-00592-x.
15. Koteluk O, Wartecki A, Mazurek S, Kolodziejczak I, Mackiewicz A. How do machines learn? Artificial intelligence as a new era in medicine. J Pers Med. 2021;11(1). PMID: 33430240. doi: 10.3390/jpm11010032.
16. Oh MY, Kim HS, Jung YM, Lee HC, Lee SB, Lee SM. Machine learning-based explainable automated nonlinear computation scoring system for health score and an application for prediction of perioperative stroke: Retrospective study. J Med Internet Res. 2025;27:e58021. PMID: 40106818. doi: 10.2196/58021.
17. Han R, Liu CB, Sun W, Yu S, Zheng H, Deng L. Machine learning of automatic hierarchical multi-label classification method for identifying metal failure mechanisms. Sci Rep. 2025;15(1):19904. PMID: 40481146. doi: 10.1038/s41598-025-05076-z.
18. Hein ZM, Guruparan D, Okunsai B, Che Mohd Nassir CMN, Ramli MDC, Kumar S. AI and machine learning in biology: From genes to proteins. Biology (Basel). 2025;14(10). PMID: 41154856. doi: 10.3390/biology14101453.
19. Sadr H, Nazari M, Khodaverdian Z, Farzan R, Yousefzadeh-Chabok S, Ashoobi MT, et al. Unveiling the potential of artificial intelligence in revolutionizing disease diagnosis and prediction: a comprehensive review of machine learning and deep learning approaches. Eur J Med Res. 2025;30(1):418. PMID: 40414894. doi: 10.1186/s40001-025-02680-7.
20. Fountzilas E, Pearce T, Baysal MA, Chakraborty A, Tsimberidou AM. Convergence of evolving artificial intelligence and machine learning techniques in precision oncology. NPJ Digit Med. 2025;8(1):75. PMID: 39890986. doi: 10.1038/s41746-025-01471-y.
21. Al-Qudimat AR, Fares ZE, Elaarag M, Osman M, Al-Zoubi RM, Aboumarzouk OM. Advancing medical research through artificial intelligence: Progressive and transformative strategies: A literature review. Health Sci Rep. 2025;8(2):e70200. PMID: 39980823. doi: 10.1002/hsr2.70200.
22. Pinto-Coelho L. How artificial intelligence is shaping medical imaging technology: A survey of innovations and applications. Bioengineering (Basel). 2023;10(12). PMID: 38136026. doi: 10.3390/bioengineering10121435.

23. Athanasopoulou K, Michalopoulou VI, Scorilas A, Adamopoulos PG. Integrating artificial intelligence in next-generation sequencing: Advances, challenges, and future directions. Curr Issues Mol Biol. 2025;47(6). PMID: 40699869. doi: 10.3390/cimb47060470.
24. Wu Y, Xie L. AI-driven multiomics integration for multiscale predictive modeling of genotype-environment-phenotype relationships. Comput Struct Biotechnol J. 2025;27:265–277. PMID: 39886532. doi: 10.1016/j.csbj.2024.12.030.
25. Abedi A, Verma A, Jain D, Kaetheeswaran J, Chui C, Lankarany M, et al. AI-driven real-time monitoring of cardiovascular conditions with wearable devices: Scoping review. JMIR Mhealth Uhealth. 2025;13:e73846. PMID: 41218203. doi: 10.2196/73846.
26. Cohen RY, Kovacheva VP. A methodology for a scalable, collaborative, and resource-efficient platform, MERLIN, to facilitate healthcare AI research. IEEE J Biomed Health Inform. 2023;27(6):3014–3025. PMID: 37030761. doi: 10.1109/JBHI.2023.3259395.
27. Doan LMT, Shahhosseini K, Verma S, Marefat A, Locicero G, Verma S, et al. Bridging modalities with AI: a review of AI advances in multimodal biomedical imaging. Commun Eng. 2026;5(1):30. PMID: 41688558. doi: 10.1038/s44172-026-00602-x.
28. Maleki Varnosfaderani S, Forouzanfar M. The role of AI in hospitals and clinics: Transforming healthcare in the 21st century. Bioengineering (Basel). 2024;11(4). PMID: 38671759. doi: 10.3390/bioengineering11040337.
29. Shin Y, Lee M, Lee Y, Kim K, Kim T. Artificial intelligence-powered quality assurance: Transforming Diagnostics, surgery, and patient care-innovations, limitations, and future directions. Life (Basel). 2025 ;15(4). PMID: 40283208. doi: 10.3390/life15040654.
30. All of Us Research Program Genomics I. Genomic data in the All of Us Research Program. Nature. 2024;627(8003):340–346. PMID: 38374255. doi: 10.1038/s41586-023-06957-x.
31. Moreno M, Vilaca R, Ferreira PG. Scalable transcriptomics analysis with Dask: applications in data science and machine learning. BMC Bioinformatics. 2022 Nov 30;23(1):514. PMID: 36451115. doi: 10.1186/s12859-022-05065-3.
32. Johnson KB, Wei WQ, Weeraratne D, Frisse ME, Misulis K, Rhee K, et al. Precision medicine, AI, and the future of personalized health care. Clin Transl Sci. 2021;14(1):86–93. PMID: 32961010. doi: 10.1111/cts.12884.
33. Faiyazuddin M, Rahman SJQ, Anand G, Siddiqui RK, Mehta R, Khatib MN, et al. The impact of artificial intelligence on healthcare: A Comprehensive review of advancements in diagnostics, treatment, and operational efficiency. Health Sci Rep. 2025;8(1):e70312. PMID: 39763580. doi: 10.1002/hsr2.70312.
34. Zhang M, Zheng Y, Maidaiti X, Liang B, Wei Y, Sun F. Integrating Machine Learning into Statistical Methods in Disease Risk Prediction Modeling: A Systematic Review. Health Data Sci. 2024;4:0165. PMID: 39050273. doi: 10.34133/hds.0165.
35. Mariani MC, Tweneboah OK, Bhuiyan MAM. Supervised machine learning models applied to disease diagnosis and prognosis. AIMS Public Health. 2019;6(4):405–423. PMID: 31909063. doi: 10.3934/publichealth.2019.4.405.
36. Fahim YA, Hasani IW, Kabba S, Ragab WM. Artificial intelligence in healthcare and medicine: clinical applications, therapeutic advances, and future perspectives. Eur J Med Res. 2025;30(1):848. PMID: 40988064. doi: 10.1186/s40001-025-03196-w.
37. Bjerre LM, Peixoto C, Alkurd R, Talarico R, Abielmona R. Comparing AI/ML approaches and classical regression for predictive modeling using large population health databases: Applications to COVID-19 case prediction. Glob Epidemiol. 2024;8:100168. PMID: 39435397. doi: 10.1016/j.gloepi.2024.100168.
38. Chowdhury MZI, Leung AA, Walker RL, Sikdar KC, O'Beirne M, Quan H, et al. A comparison of machine learning algorithms and traditional regression-based statistical modeling for predicting hypertension incidence in a Canadian population. Sci Rep. 2023;13(1):13. PMID: 36593280. doi: 10.1038/s41598-022-27264-x.
39. Verbeeck N, Caprioli RM, Van de Plas R. Unsupervised machine learning for exploratory data analysis in imaging mass spectrometry. Mass Spectrom Rev. 2020;39(3):245–291. PMID: 31602691. doi: 10.1002/mas.21602.
40. Eckhardt CM, Madjarova SJ, Williams RJ, Ollivier M, Karlsson J, Pareek A, et al. Unsupervised machine learning methods and emerging applications in healthcare. Knee Surg Sports Traumatol Arthrosc. 2023;31(2):376–381. PMID: 36378293. doi: 10.1007/s00167-022-07233-7.
41. Yan J, Risacher SL, Shen L, Saykin AJ. Network approaches to systems biology analysis of complex disease: integrative methods for multi-omics data. Brief Bioinform. 2018;19(6):1370–1381. PMID: 28679163. doi: 10.1093/bib/bbx066.
42. Ghasemi A, Hosseinpanah F, Kashfi K, Bahadoran Z. Research Hypothesis: A Brief History, Central Role in Scientific Inquiry, and Characteristics. Addict Health. 2025 Jan;17:1623. PMID: 41113946. doi: 10.34172/ahj.1623.
43. Yarborough M. Moving towards less biased research. BMJ Open Sci. 2021;5(1):e100116. PMID: 35047699. doi: 10.1136/bmjos-2020-100116.
44. Kiester L, Turp C. Artificial intelligence behind the scenes: PubMed's Best Match algorithm. J Med Libr Assoc. 2022;110(1):15–22. PMID: 35210958. doi: 10.5195/jmla.2022.1236.
45. Simon BD, Ozyoruk KB, Gelikman DG, Harmon SA, Turkbey B. The future of multimodal artificial intelligence models for integrating imaging and clinical metadata: a narrative review. Diagn Interv Radiol. 2025;31(4):303–312. PMID: 39354728. doi: 10.4274/dir.2024.242631.
46. Meng X. Cross-domain information fusion and personalized recommendation in artificial intelligence recommendation system based on mathematical matrix decomposition. Sci Rep. 2024;14(1):7816. PMID: 38570539. doi: 10.1038/s41598-024-57240-6.
47. Gao S, Fang A, Huang Y, Giunchiglia V, Noori A, Schwarz JR, et al. Empowering biomedical discovery with AI agents. Cell. 2024;187(22):6125-6151. PMID: 39486399. doi: 10.1016/j.cell.2024.09.022.
48. Holland I, Davies JA. Automation in the life science research laboratory. Front Bioeng Biotechnol. 2020;8:571777. PMID: 33282848. doi: 10.3389/fbioe.2020.571777.
49. Madanchian M, Taherdoost H. The impact of artificial intelligence on research efficiency. Results in Engineering. 2025;26:104743. doi: 10.1016/j.rineng.2025.104743.
50. Nisa U, Shirazi M, Saip MA, Syafiq M, Pozi M. Agentic AI: The age of reasoning—A review. J Automation intelligence. 2025:in press. doi: 10.1016/j.jai.2025.08.003.
51. Feng J, Phillips RV, Malenica I, Bishara A, Hubbard AE, Celi LA, et al. Clinical artificial intelligence quality improvement: towards continual monitoring and updating of AI algorithms in healthcare. NPJ Digit Med. 2022;5(1):66. PMID: 35641814. doi: 10.1038/s41746-022-00611-y.
52. Ulvi Saygi Ayvaci M, Jacobi VS, Ryu Y, Gundreddy SPS, Tanriover B. Clinically guided adaptive machine learning update strategies for predicting severe COVID-19 outcomes. Am J Med. 2025; 138(2):228-235 e1. PMID: 39424215. doi: 10.1016/j.amjmed.2024.10.011.

53. Hao X, Demir E, Eyers D. Beyond human-in-the-loop: Sensemaking between artificial intelligence and human intelligence collaboration. Sustainable Futures. 2025;10(1):101152. doi: 10.1016/j.sftr.2025.101152.
54. Greengrass CJ. Transforming clinical reasoning-the role of AI in supporting human cognitive limitations. Front Digit Health. 2025;7:1715440. PMID: 41561162. doi: 10.3389/fdgth.2025.1715440.
55. Mexico Gd. National Health Institutes of México: Instituto Nacional de Perinatología Isidro Espinosa de los Reyes Mexico City, Mexico: Gobierno de Mexico; 2026 [Available from: https://www.gob.mx/salud/inper].
56. Morales-Suárez M. Professional career of Dr. Eduardo Jurado García (1921-1998). An approach to his life and work. Perinatol Reprod Hum. 2010;24(3):207–211.
57. Pratasevich T, Yankouskaya N, Dzianisik N, Onehina V, Palkhouskaya A. Clinical and laboratory features of neonatal seizures in Belarus. Newborn (Clarksville, Md). 2026;5(1):6–12. doi: 10.5005/jp-journals-11002-0154.
58. Government-of-Belarus. Healthcare Ministry: Belarus has one of world's lowest infant mortality rates Minsk, Belarus: Healthcare Ministry of Belarus; 2026 [Available from: https://www.belarus.by/en/press-center/press-release/healthcare-ministry-belarus-has-one-of--worlds-lowest-infant-mortality-rates_i_0000200088.html].
59. Singh S, Kaushal M, Guaragni B, et al. Systematized Systemic Sonographic Surveillance (S4). Newborn 2025;4(4):181–193. DOI: 10.5005/jp-journals-11002-0142.
60. Kaushal M, Balla KC. Clinical Imaging: Point-of-care ultrasound for endotracheal tube localization in neonates: Why it matters. Newborn (Clarksville, Md). 2026;5(1):58–60. doi: 10.5005/jp-journals-11002-0141.
61. Frydrysiak-Brzozowska A, Singh S, Adamczyk-Gruszka O, Piórkowska K, Jóźwik A. Impact of the Ukrainian conflict on mothers and young infants who have had to migrate to Poland. Newborn (Clarksville, Md). 2026;5(1):26–38. doi: 10.5005/jp-journals-11002-0151.
62. UNHCR. Ukraine emergency Geneva, Switzerland: United Nations High Commissioner for Refugees (UN Refugee Agency); 2026 [Available from: https://www.unhcr.org/us/emergencies/ukraine-emergency].
63. Beitsch R. UN says more than 2.5 million refugees have left Ukraine Washington, D.C., USA: The Hill; 2022 [Available from: https://thehill.com/policy/international/597818-un-says-more-than-25-million-refugees-have-left-ukraine/].
64. Duszczyk M, Kaczmarczyk P. The War in Ukraine and Migration to Poland: Outlook and challenges Hamburg, Germany: ZBW – Leibniz Information Centre for Economics with CEPS – Centre for European Policy Studies; 2022 [Available from: https://www.intereconomics.eu/contents/year/2022/number/3/article/the-war-in-ukraine-and-migration-to-poland-outlook-and-challenges.html].
65. Jankowski M, Lazarus JV, Kuchyn I, Zemskov S, Galazkowski R, Gujski M. One Year On: Poland's Public Health Initiatives and National Response to Millions of Refugees from Ukraine. Med Sci Monit. 2023;29:e940223. PMID: 36998206. doi: 10.12659/MSM.940223.
66. Maheshwari A, Mohammadabadi T, Michie C. Calprotectin: An Overview. Newborn (Clarksville, Md). 2026;5(1):39–57. doi: 10.5005/jp-journals-11002-0153.
67. Carnazzo V, Redi S, Basile V, Natali P, Gulli F, Equitani F, et al. Calprotectin: two sides of the same coin. Rheumatology (Oxford). 2024;63(1):26–33. PMID: 37603715. doi: 10.1093/rheumatology/kead405.
68. Maheshwari A. Innate immune memory in macrophages. Newborn (Clarksville, Md). 2023;2(1):60–79. doi: 10.5005/jp-journals-11002-0058.
69. Mezu-Ndubuisi OJ, Maheshwari A. Role of macrophages in fetal development and perinatal disorders. Pediatr Res. 2021;90(3):513–523. PMID: 33070164. doi: 10.1038/s41390-020-01209-4.
70. Ho TTB, Groer MW, Kane B, Yee AL, Torres BA, Gilbert JA, et al. Enteric dysbiosis and fecal calprotectin expression in premature infants. Pediatr Res. 2019;85(3):361–368. PMID: 30631136. doi: 10.1038/s41390-018-0254-y.
71. Azramezani Kopi T, Shahrokh S, Mirzaei S, Asadzadeh Aghdaei H, Amini Kadijani A. The role of serum calprotectin as a novel biomarker in inflammatory bowel diseases: a review study. Gastroenterol Hepatol Bed Bench. 2019 Summer;12(3):183–189. PMID: 31528300.
72. Mestrovic A, Perkovic N, Bozic D, Kumric M, Vilovic M, Bozic J. Precision medicine in inflammatory bowel disease: A spotlight on emerging molecular biomarkers. Biomedicines. 2024;12(7). PMID: 39062093. doi: 10.3390/biomedicines12071520.
73. Khare AK. Meckel diverticulum. Newborn (Clarksville, Md). 2026;5(1):13–19. doi: 10.5005/jp-journals-11002-0149.
74. St-Vil D, Brandt ML, Panic S, Bensoussan AL, Blanchard H. Meckel's diverticulum in children: a 20-year review. J Pediatr Surg. 1991;26(11):1289–1292. PMID: 1812259. doi: 10.1016/0022-3468(91)90601-o.
75. Fusco JC, Achey MA, Upperman JS. Meckel's diverticulum: Evaluation and management. Semin Pediatr Surg. 2022;31(1):151142. PMID: 35305798. doi: 10.1016/j.sempedsurg.2022.151142.
76. Huang CC, Lai MW, Hwang FM, Yeh YC, Chen SY, Kong MS, et al. Diverse presentations in pediatric Meckel's diverticulum: a review of 100 cases. Pediatr Neonatol. 2014;55(5):369–375. PMID: 24685339. doi: 10.1016/j.pedneo.2013.12.005.
77. DiGiacomo JC, Cottone FJ. Surgical treatment of Meckel's diverticulum. South Med J. 1993 ;86(6):671–675. PMID: 8506491. doi: 10.1097/00007611-199306000-00017.
78. Maheshwari A, Das JK, Guaragni B, Nimbalkar S, Alghnimei N, Alallah J, et al. Principal component analysis: Progress and applications. Newborn (Clarksville, Md). 2026;5(1):20–25. doi: 10.5005/jp-journals-11002-0150.
79. Jolliffe IT, Cadima J. Principal component analysis: a review and recent developments. Philos Trans A Math Phys Eng Sci. 2016;374(2065):20150202. PMID: 26953178. doi: 10.1098/rsta.2015.0202.
80. Johnstone IM, Lu AY. On consistency and sparsity for principal components analysis in high dimensions. J Am Stat Assoc. 2009;104(486):682–693. PMID: 20617121. doi: 10.1198/jasa.2009.0121.
81. Rao AG. Radiology case reports: Midgut malrotation without volvulus. Newborn (Clarksville, Md). 2026;5(1):61–64. doi: 10.5005/jp-journals-11002-0148.
82. Ford EG, Senac MO, Jr., Srikanth MS, Weitzman JJ. Malrotation of the intestine in children. Ann Surg. 1992;215(2):172–178. PMID: 1546904. doi: 10.1097/00000658-199202000-00013.
83. Martin V, Shaw-Smith C. Review of genetic factors in intestinal malrotation. Pediatr Surg Int. 2010;26(8):769–781. PMID: 20549505. doi: 10.1007/s00383-010-2622-5.
84. Welsh IC, Thomsen M, Gludish DW, Alfonso-Parra C, Bai Y, Martin JF, et al. Integration of left-right PITX2 transcription and Wnt signaling drives asymmetric gut morphogenesis via Daam2. Dev Cell. 2013 ;26(6):629–644. PMID: 24091014. doi: 10.1016/j.devcel.2013.07.019.

85. Singh S, Maneval ML, Sargar K, Kulaylat AN. Malrotation of the gut: Not Just a diagnosis of infancy. Cureus. 2025;17(4):e82229. PMID: 40370924. doi: 10.7759/cureus.82229.
86. Yang X, Wang W, Wang K, Zhao J, Sun L, Jiang S, et al. Identification and treatment of intestinal malrotation with midgut volvulus in childhood: a multicenter retrospective study. Front Pediatr. 2024;12:1390856. PMID: 38803636. doi: 10.3389/fped.2024.1390856.
87. Michie C, Maheshwari A. Searching, a rapidly evolving skill. Newborn (Clarksville, Md). 2026;5(1):1–5. doi: 10.5005/jp-journals-11002-0152.
88. Maheshwari A, Huisman TAGM. Artificial intelligence in newborn medicine. Newborn (Clarksville, Md). 2024;3(2):96–110. doi: 10.5005/jp-journals-11002-0095.
89. Chevalier A, Dosso C. The influence of medical expertise and information search skills on medical information searching: Comparative analysis from a free data set. JMIR Form Res. 2025;9:e62754. PMID: 40245399. doi: 10.2196/62754.
90. Nelson HC, Beauchamp MT, Pace AA. The reliability gap: How traditional search engines outperform artificial intelligence (ai) chatbots in Rosacea Public Health Information Quality. Cureus. 2025 ;17(6):e86543. PMID: 40698243. doi: 10.7759/cureus.86543.
91. Van Seggelen-Damen ICM, Van Hezewijk R, Helsdingen AS, Wopereis I. Reflection: A Socratic approach. Theory Psychol. 2017;27(6):793–814. PMID: 29249867. doi: 10.1177/0959354317736388.
92. Ho YR, Chen BY, Li CM. Thinking more wisely: using the Socratic method to develop critical thinking skills amongst healthcare students. BMC Med Educ. 2023;23(1):173. PMID: 36941597. doi: 10.1186/s12909-023-04134-2.
93. Chen X, Xiao L. Serendipitous sparks: AI information encounter, cognitive flexibility, AI literacy, and university student creativity. Front Psychol. 2025;16:1623730. PMID: 41383430. doi: 10.3389/fpsyg.2025.1623730.
94. Belisle-Pipon JC. Why we need to be careful with LLMs in medicine. Front Med (Lausanne). 2024;11:1495582. PMID: 39697212. doi: 10.3389/fmed.2024.1495582.
95. Maity S, Saikia MJ. Large language models in healthcare and medical applications: A Review. Bioengineering (Basel). 2025;12(6). PMID: 40564447. doi: 10.3390/bioengineering12060631.
96. Ebenebe CU, Schriever K, Apostolidou S, Wolf M, Herrmann J, Singer D, et al. Recommendations for endotracheal tube insertion depths in children. Emerg Med J. 2023;40(8):583–587. PMID: 37336629. doi: 10.1136/emermed-2022-212494.
97. Schmolzer GM, O'Reilly M, Davis PG, Cheung PY, Roehr CC. Confirmation of correct tracheal tube placement in newborn infants. Resuscitation. 2013;84(6):731–737. PMID: 23211476. doi: 10.1016/j.resuscitation.2012.11.028.
98. Phoenixmed-at-arizona.edu. Ling He Phoenix, Arizona, United States: University of Arizona; 2025 [Available from: https://phoenixmed.arizona.edu/directory/he-ling].
99. Sabreeenrichmentacademy.org. Sonji-Fatima St. Louis, Missouri, United States: Sabree Enrichment Academy, St. Louis, United States; 2025 [Available from: https://www.drsonjifatima.com//.
100. Collegium Medicum P, Poland. Office of the Dean, Faculty of Health Sciences, Płock, Poland Płock, Poland: Collegium Medicum, Płock, Poland; 2025 [Available from: https://mazowiecka.edu.pl/en/collegium-medicum/].

COMMENTARY

Searching, a Rapidly Evolving Skill

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Received on: 21 January 2026; *Accepted on:* 24 February 2026; *Published on:* 08 April 2026

Abstract

With the advent of artificial intelligence (AI) neural networks, their capacity to deliver answers to searches for medical or scientific information is rapidly taking over from search engines and publishers. This should empower searches based on thoughtful questions, including "why," "how," and "what if" – the Socratic approach. Outputs from AI large language models (LLMs) require careful validation, and it is likely they will limit serendipity.

Keywords: Agentic AI systems, Alexa, Algorithmic biases, Anthropic's Claude, Creativity, Fabrications, Filter bubbles, Hallucinations, Health information seeking behavior, Index Medicus, Infant, Large language models, Mothers, National Library of Medicine, Newborn, Occam's razor, OpenAI's Chat GPT, Personalized algorithms, PubMed, Searching, Serendipity, Siri, Socratic questions, Team consultations, Voice assistants.

Newborn (2026): 10.5005/jp-journals-11002-0152

Key Points

- Searching for information online relating to health is moving onto artificial intelligence (AI) platforms.
- Artificial intelligence is likely to deliver most search results to the public and professionals in the future.
- Caution is required when employing AI systems because their outcomes require validation.
- We need to understand better how our patients use their personal healthcare search results.
- Artificial intelligence's personalization will change how we search.

We share a common habit.[1,2] We often question, explore, and search for answers[3,4] using a mobile, laptop, or vocal assistant.[5,6] This core pursuit, searching for health information, is changing dramatically, search processes are becoming volatile.[7–9] "Traditional" search engine traffic is falling and publishers predict this decrease will continue.[10,11] Search engines are being overtaken by a range of AI systems. Assistants such as Siri or Alexa, OpenAI's Chat GPT, and Anthropic Claude can provide answers vocally or written, to help everyone search.[12,13] ChatGPT is possibly the fastest growing application ever, now with over 800 million users each week.[10,14]

The printed page transformed societies in Western Europe steadily from the 15th century.[15] Large scale printing disseminated new skills of reading and calculation, literacies, and knowledge.[16] It generated novel patterns of enquiry, teaching, and writing.[16,17] However, this did not improve the impact for all researchers.[18,19] Robert Hooke wrote in 1679: "Twill be much better to embrace the influence of Providence."[20] In 1879, "Index Medicus" was launched by the National Library of Medicine, compiled manually for a monthly publication.[21] Medical textbooks and journals became progressively more affordable, popular, and routine occupants of a clinician's office.[19] Museums, radio, television and newspapers recorded, documented, indexed, and curated archives that enriched searching the fields of health and social care.[22] Patients and potential patients used all these too to seek out information about their health.[23] In the 1980s, their telephone calls to the newly opened Cancer Information Service were studied, before internet searches were available.[24–26] Freimuth and colleagues outlined a pentagonal frame of determinants for search: Prior knowledge;

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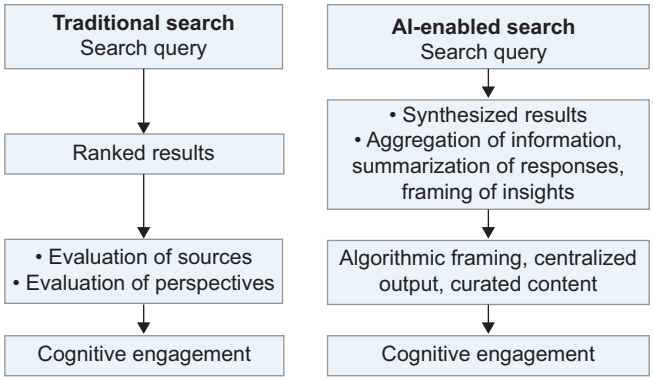

Fig. 1: Differences between traditional and AI-enabled search systems. The figure contrasts searching with conventional processes with AI-enabled paradigms. In traditional search, users submit a query and receive a ranked list of possible answers, which require evaluation of sources and perspectives, followed by manual synthesis of information for knowledge construction. In contrast, AI-enabled search systems generate a synthesized response by aggregating and summarizing information from diverse data sources (LLMs) before tailoring it and presenting it to the user. This approach reduces cognitive load and may increase efficiency, but it centralizes information curation, framing, and prioritization employing an unknown algorithm. This comparison highlights the shifts in information access, exposure to diverse viewpoints, and perceived epistemic authority in AI-mediated environments

understanding of goals and time frames; cost-benefit analysis of search; actual undertaking of the search; evaluation of the information for adequacy, credibility, and applicability; and finally, appraisal of the decision as to its adequacy.[27–29]

Following the launch of the internet, "PubMed" replaced "Index Medicus" as an online resource.[30] Today, "PubMed" hosts millions of general medical searches through its website each day, as well as automated traffic searches.[31,32] It houses approximately 36 million peer-reviewed articles, increasing by approximately a million a year.[33] In addition, clinicians search using a diversity of other search engines including intelligent textbooks, best-practice journals, national guidelines, and specialized resources from Universities, Colleges and Societies.[31,34]

Artificial intelligence search systems are delivering evolutionary, revolutionary transformations (Fig. 1).[35] Large language models (LLMs), trained with self-supervised machine learning from vast numbers of documents reduce unnecessary browsing, guiding greater focus.[36,37] Indeed the stated aim of Google Zero is to reduce the number of clicks you would normally employ by providing you with a single instant synthesis, a complete answer.[38] This limitation means rigorous searchers may require several separate questions to examine evidence or apply critical thinking in diverse ways to their searches.[39–40]

Some users describe AI chatbots as supporting their creativity, just as in simulation training.[39] Specialized AI searches can be tailored to work in the areas of evidence-based practice, genomics, or by linking concepts and mechanisms to explore connections.[33,39] The "Consensus" AI system offers to search 250 million peer-reviewed articles with powerful tools to provide evidence-based summaries and citations.[40] In the care of newborn infants, AI holds promise as a research tool in the searching and analysis of vast amounts of data that could be collected from continuous monitoring.[41,42]

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How to cite this article: Michie C, Maheshwari A. Searching, a Rapidly Evolving Skill. Newborn 2026;5(1):1–5.

Source of support: Nil

Conflict of interest: None

Digital provenance is required before relying on the results of AI searches.[43] Although we no longer require the "pleases," "thank-you's" or promises of a lunch out – as were once needed for medical school librarians – AI outputs require careful checking.[44] Incorrect responses or fabrications are not always detectable. Large language models can produce incorrect responses or fabrications, often referred to as hallucinations.[34,45] Racial, socioeconomic, and sexual biases can influence search outputs.[46] There are many geographic disparities too. Large language models have a richer training background in the West but are still being developed in the peri-equatorial/tropical economies such as in Africa or India, regions with larger numbers of mothers and infants.[47] India's strengths in computational software systems may bring global solutions to this issue.[48,49] Artificial intelligence bots collect information continuously as they trawl the internet; the realities and limitations of these inputs cannot be ignored.[50,51]

Artificial intelligence systems are widely used by patients, applying strategies similar to those used with telephone services forty years ago: Social habits have persisted across a generation![52,53] Some very human-centered techniques can be observed.[54] Users typically employ multiple search tools, applying those outcomes that best match their perception of their situations, time-frames, and understanding. Suboptimal advice may be preferred and applied, even though the source technology is not understood.[55,56] Gratifyingly, it seems people still prefer to speak to a human professional regarding their health and that of their infants. However, it is conceivable that in future more approachable or convenient vocal AI systems, for instance, could challenge this norm.[57,58]

Artificial intelligence-enabled searches may subtly change our intellectual environments by removing serendipity.[59] In the past many scientific discoveries have been the result of unexpected "accidents." Now it might be more likely for searchers to uncover unexpected, but beneficial material (Fig. 2).[60] We might not fortuitously run into a useful title when browsing for a book on a shelf. Or perhaps derive inspiration from the page next to the targeted one in an encyclopedia or website. Personalized algorithms designed to target information to us based on our internet use can inadvertently create intellectual "filter bubbles" that restrict the diversity of outcomes provided to a researcher.[61] Will personalized AI searches and workflows limit our "Eureka" moments? Will we end up rationing the providence commended by Hooke, or limiting a neonatologist's application of Occam's razor?[62]

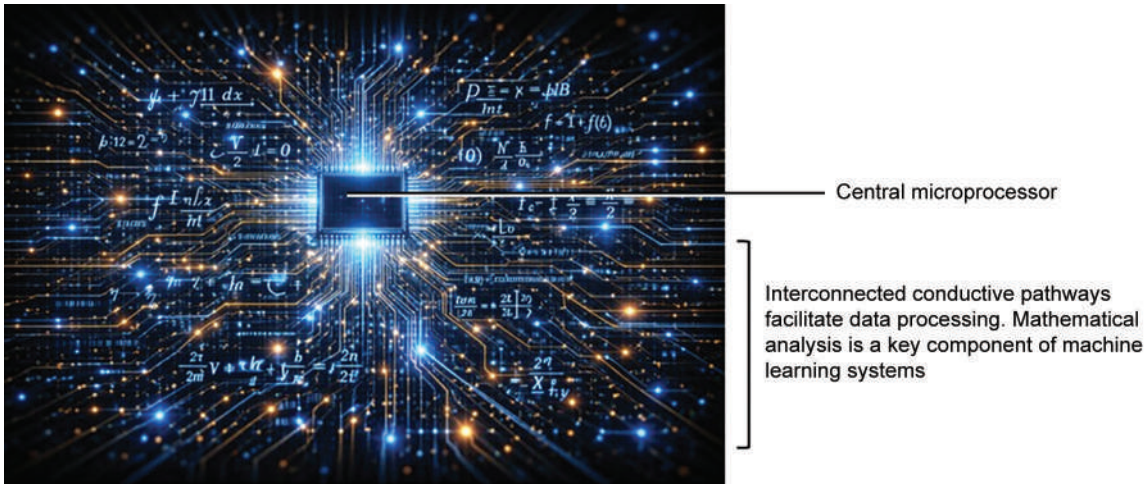

Fig. 2: Schematic representation of an AI–inspired computational circuit. In AI hardware, a central microprocessor is often embedded within an intricate network of conductive pathways, which facilitate data flow for information processing. Artificial intelligence is fundamentally built upon mathematical expressions and analytical formulas that enable machines to process data, recognize patterns, and make informed decisions. Core mathematical disciplines such as linear algebra, calculus, probability, and statistics form the backbone of AI algorithms. Matrices and vectors are used to represent data and neural network parameters, calculus for optimization through techniques like gradient descent, and probability theory-based inferences in predictive modeling and estimation of uncertainty. Analytical formulas can be used to define loss functions, activation functions, and model evaluation metrics, allowing systems to iteratively adjust parameters and improve performance. Together, these mathematical foundations make AI capable of learning from data, generalizing patterns, and solving complex real-world problems. For instance, to prepare this figure we first drafted a legend and then fed serial versions with minor modifications into ChatGPT (OpenAI 2026. ChatGPT version 5. https://chat.openai.com) until a desired illustration was obtained

Artificial intelligence tools are powerful, speedy, appealing, and even seductive.[63] To apply them effectively to searches we need to think, review, and modify our personal exploring habits, pushing back on over-simpifications.[64] Joint consultations within clinical groups, for example using Socratic, open-ended questions, can help develop secret sauces; methods for searches. Teams are stronger than an individual for hunting or tracking! Our researches, care of patients and clinical services must not be run, or overrun, by expanding AI agents.[65]

References

1. Grundmann CC, Arndt VA, Ebrahimi C, et al. Studying human habit formation through motor sequence learning. Cogn Affect Behav Neurosci 2025;25(4):941–951. DOI: 10.3758/s13415-025-01300-5.
2. Christodoulou A, Roussos P. "Phone in the room, mind on the Roam": Investigating the impact of mobile phone presence on distraction. Eur J Investig Health Psychol Educ 2025;15(5):74. DOI: 10.3390/ejihpe15050074.
3. Herbert M. The Science of Curiosity: Why Humans Love to Explore and Discover. Sydney, New South Wales, Australia: CMYCubes.com; 2025. Available from: https://www.cmycubes.com/blogs/cmycubes/the-science-of-curiosity.
4. Haroon M, Phillips R. "There is nothing like looking, if you want to find something" – Asking questions and searching for answers – The evidence based approach. Arch Dis Child Educ Pract Ed 2010;95(2):34–39. DOI: 10.1136/adc.2009.161570.
5. Alagha EC, Helbing RR. Evaluating the quality of voice assistants' responses to consumer health questions about vaccines: An exploratory comparison of Alexa, Google Assistant and Siri. BMJ Health Care Inform 2019;26(1):e100075. DOI: 10.1136/bmjhci-2019-100075.
6. Bach RL, Wenz A. Studying health-related internet and mobile device use using web logs and smartphone records. PLoS One 2020;15(6):e0234663. DOI: 10.1371/journal.pone.0234663.
7. Diviani N. On the centrality of information appraisal in health literacy research. Health Lit Res Pract 2019;3(1):e21–e24. DOI: 10.3928/24748307-20181214-01.
8. Arruda RM, Ayoub IA, Nunes R, et al. Consulting "Dr. Google": How the digital search for internet health information influences doctor-patient relationship. Cad Saude Publica 2025;41(7):e00153623. DOI: 10.1590/0102-311XEN153623.
9. Yun HS, Bickmore T. Online health information-seeking in the era of large language models: Cross-sectional web-based survey study. J Med Internet Res 2025;27:e68560. DOI: 10.2196/68560.
10. Newman N. Journalism, Media, and Technology Trends and Predictions 2026. Oxford, United Kingdom: Thomson Reuters Foundation; 2026. Available from: https://reutersinstitute.politics.ox.ac.uk/journalism-media-and-technology-trends-and-predictions-2026.
11. Zul. News Sites Lose Traffic as Google AI Overviews Replace Search. Bristol, United Kingdom: MarketingTech; 2025. Available from: https://www.marketingtechnews.net/news/news-sites-lose-traffic-as-google-ai-overviews-replace-traditional-search/.
12. Wardle C, Urbani S, Wang E. Evolving health information-seeking behavior in the context of Google AI overviews, ChatGPT, and Alexa: Interview study using the think-aloud protocol. J Med Internet Res 2025;27:e79961. DOI: 10.2196/79961.
13. Wang X, Cohen RA. Health Information Technology Use Among Adults: United States, July–December 2022. Atlanta, Georgia, United States: National Center for Health Statistics; 2022. Available from: https://stacks.cdc.gov/view/cdc/133700.
14. Bellan R. Sam Altman Says ChatGPT has Hit 800M Weekly Active Users. San Francisco, California, United States: TechCrunch; 2025. Available from: https://techcrunch.com/2025/10/06/sam-altman-says-chatgpt-has-hit-800m-weekly-active-users/.
15. Tulchinsky TH, Varavikova EA. A History of Public Health, 3rd edn. San Diego, California, United States: Elsevier, Academic Press; 2014. p. 632.
16. Kanchan S, Gaidhane A. Print media role and its impact on public health: A narrative review. Cureus 2024;16(5):e59574. DOI: 10.7759/cureus.59574.

17. Chetail F. Reading books: The positive impact of print exposure on written word recognition. Cognition 2024;251:105905. DOI: 10.1016/j.cognition.2024.105905.
18. Ossom Williamson P, Minter CIJ. Exploring PubMed as a reliable resource for scholarly communications services. J Med Libr Assoc 2019;107(1):16–29. DOI: 10.5195/jmla.2019.433.
19. Giguere A, Zomahoun HTV, Carmichael PH, et al. Printed educational materials: Effects on professional practice and healthcare outcomes. Cochrane Database Syst Rev 2020;8(8):CD004398. DOI: 10.1002/14651858.CD004398.pub4.
20. Merton RK, Barber E. The travels and adventures of serendipity: A study in sociological semantics and the sociology of science. Princeton, New Jersey, United States: Princeton University Press; 1958. p.230.
21. National-Library-of-Medicine. MEDLINE History. Bethesda, Maryland, United States: National Library of Medicine; 2024. Available from: https://www.nlm.nih.gov/medline/medline_history.html.
22. Tunc U. Museums of medicine and health: Curating public health. Infect Dis Clin Microbiol 2023;5(1):69–81. DOI: 10.36519/idcm.2023.195.
23. Tetro JA. Learning from history to increase positive public reception and social value alignment of evidence-based science communication. J Microbiol Biol Educ 2018;19(1):19.1.41. DOI: 10.1128/jmbe.v19i1.1532.
24. Anderson DM, Duffy K, Hallett CD, et al. Cancer prevention counseling on telephone helplines. Public Health Rep 1992;107(3):278–283. PMID: 1594737.
25. Yavari B, Kolbehdari N, Gann L, et al. The digital health revolution: Exploring the impact of online cancer information on self-reported preventive behaviors. medRxiv 2024. DOI: 10.1101/2024.05.20.24307517.
26. Erfani SS, Abedin B. Effects of web based cancer support resources use on cancer affected people: A systematic literature review. Int Technol Manag Rev 2014;4(4):201–211. DOI: 10.2991/itmr.2014.4.4.4.
27. National-Cancer-Institute. Making Health Communication Programs Work. Atlanta, Georgia, United States: National Institutes of Health, National Cancer Institute; 2002.
28. DeLorme DE, Huh J, Reid LN. Source selection in prescription drug information seeking and influencing factors: Applying the comprehensive model of information seeking in an American context. J Health Commun 2011;16(7):766–787. DOI: 10.1080/10810730.2011.561914.
29. Freimuth VS, Stein JA, Kean TJ. Searching for Health Information: The Cancer Information Service Model. Pennsylvania, Philadelphia, United States: University of Pennsylvania Press; 1989. pp. 1–249.
30. NCBI. About PubMed. Bethesda, Maryland, United States: National Center for Biotechnology Information (NCBI); 1996. Available from: https://pubmed.ncbi.nlm.nih.gov/about/.
31. Lindsey WT, Olin BR. PubMed searches: Overview and strategies for clinicians. Nutr Clin Pract 2013;28(2):165–176. DOI: 10.1177/0884533613475821.
32. Rajit D, Du L, Teede H, et al. Automated citation searching in systematic review production: A simulation study. Res Synth Methods 2025;16(1):211–227. DOI: 10.1017/rsm.2024.15.
33. Jin Q, Leaman R, Lu Z. PubMed and beyond: Biomedical literature search in the age of artificial intelligence. EBioMedicine 2024;100:104988. DOI: 10.1016/j.ebiom.2024.104988.
34. Walters WH. Comparing conventional and alternative mechanisms of discovering and accessing the scientific literature. Proc Natl Acad Sci U S A 2025;122(27):e2503051122. DOI: 10.1073/pnas.2503051122.
35. Zybailov BL, Kosovsky GY, Glazko GV, et al. Evolutionary perspectives on human-artificial intelligence convergence. Acta Naturae 2024;16(3):4–17. DOI: 10.32607/actanaturae.27406.
36. Stadler M, Bannert M, Sailer M. Cognitive ease at a cost: LLMs reduce mental effort but compromise depth in student scientific inquiry. Computers Hum Behav 2024;160:108386. DOI: 10.1016/j.chb.2024.108386.
37. Yu E, Chu X, Zhang W, et al. Large language models in medicine: Applications, challenges, and future directions. Int J Med Sci 2025;22(11):2792–2801. DOI: 10.7150/ijms.111780.
38. Startups. What is Google Zero and why does it matter? London, United Kingdom: Startups.co.uk; 2026. Available from: https://startups.co.uk/news/google-zero/.
39. Abdelhalim H, Berber A, Lodi M, et al. Artificial intelligence, healthcare, clinical genomics, and pharmacogenomics approaches in precision medicine. Front Genet 2022;13:929736. DOI: 10.3389/fgene.2022.929736.
40. Apata OE, Kwok OM, Lee YH. The use of generative artificial intelligence (AI) in academic research: A review of the consensus app. Cureus 2025;17(7):e87297. DOI: 10.7759/cureus.87297.
41. Arora T, Muhammad-Kamal H, Beam K. Preserving medical ethics in the era of artificial intelligence: Challenges and opportunities in neonatology. Semin Perinatol 2025;49(6):152100. DOI: 10.1016/j.semperi.2025.152100.
42. Halomoan Harahap T, Mansouri S, Salim Abdullah O, et al. An artificial intelligence approach to predict infants' health status at birth. Int J Med Inform 2024;183:105338. DOI: 10.1016/j.ijmedinf.2024.105338.
43. Sullivan BA, Beam K, Vesoulis ZA, et al. Transforming neonatal care with artificial intelligence: Challenges, ethical consideration, and opportunities. J Perinatol 2024;44(1):1–11. DOI: 10.1038/s41372-023-01848-5.
44. Linton AM. Emerging roles for librarians in the medical school curriculum and the impact on professional identity. Med Ref Serv Q 2016;35(4):414–433. DOI: 10.1080/02763869.2016.1220758.
45. Walters WH, Wilder EI. Fabrication and errors in the bibliographic citations generated by ChatGPT. Sci Rep 2023;13(1):14045. DOI: 10.1038/s41598-023-41032-5.
46. Marko JGO, Neagu CD, Anand PB. Examining inclusivity: The use of AI and diverse populations in health and social care: A systematic review. BMC Med Inform Decis Mak 2025;25(1):57. DOI: 10.1186/s12911-025-02884-1.
47. Zhu L, Mou W, Lai Y, et al. Language and cultural bias in AI: Comparing the performance of large language models developed in different countries on Traditional Chinese Medicine highlights the need for localized models. J Transl Med 2024;22(1):319. DOI: 10.1186/s12967-024-05128-4.
48. Inamdar N. Big tech bets big on AI – But can India keep pace in the global race? London, United Kingdom: British Broadcasting Corporation; 2025. Available from: https://www.bbc.com/news/articles/cd74gjw1j11o.
49. The-Economist. India could be a Different Kind of AI Superpower. London, United Kingdom: The Economist Group; 2025. Available from: https://www.economist.com/leaders/2025/09/18/india-could-be-a-different-kind-of-ai-superpower.
50. Morita PP, Lotto M, Kaur J, et al. What is the impact of artificial intelligence-based chatbots on infodemic management? Front Public Health 2024;12:1310437. DOI: 10.3389/fpubh.2024.1310437.
51. Jee H. Emergence of artificial intelligence chatbots in scientific research. J Exerc Rehabil 2023;19(3):139–140. DOI: 10.12965/jer.2346234.117.
52. Wilson L, Marasoiu M. The development and use of chatbots in public health: Scoping review. JMIR Hum Factors 2022;9(4):e35882. DOI: 10.2196/35882.
53. Khaw KW, Alnoor A, Al-Abrrow H, et al. Reactions towards organizational change: A systematic literature review. Curr Psychol 2022:1–24. DOI: 10.1007/s12144-022-03070-6.
54. Heracleous L, Bartunek J. Organization change failure, deep structures and temporality: Appreciating Wonderland. Hum Relat 2020;74(2):208–233. DOI: 10.1177/0018726720905361.

55. Islamaj Dogan R, Murray GC, Neveol A, et al. Understanding PubMed user search behavior through log analysis. Database (Oxford) 2009;2009:bap018. DOI: 10.1093/database/bap018.
56. Ecker ED, Skelly AC. Conducting a winning literature search. Evid Based Spine Care J 2010;1(1):9–14. DOI: 10.1055/s-0028-1100887.
57. Riedl R, Hogeterp SA, Reuter M. Do patients prefer a human doctor, artificial intelligence, or a blend, and is this preference dependent on medical discipline? Empirical evidence and implications for medical practice. Front Psychol 2024;15:1422177. DOI: 10.3389/fpsyg.2024.1422177.
58. Annenberg-Public-Policy-Center. Annenberg Public Policy Center of the University of Pennsylvania: ASAPH W24 AI and PCP Items. Philadelphia, Pennsylvania, United States: University of Pennsylvania; 2025.
59. Chen X, Xiao L. Serendipitous sparks: AI information encounter, cognitive flexibility, AI literacy, and university student creativity. Front Psychol 2025;16:1623730. DOI: 10.3389/fpsyg.2025.1623730.
60. Featherstone R, Walter M, MacDougall D, et al. Artificial intelligence search tools for evidence synthesis: Comparative analysis and implementation recommendations. Cochrane Evid Synth Methods 2025;3(5):e70045. DOI: 10.1002/cesm.70045.
61. Bhalerao C. How filter bubbles are biasing your opinions on social media: Filter bubbles and Echo chambers: How social media is biasing our opinions and the role of AI in it. San Francisco, California, United States: Medium.com; 2023. Available from: https://medium.com/data-and-beyond/how-filter-bubbles-are-biasing-your-opinions-on-social-media-9469b940154.
62. Cherington M. Sherlock Holmes: Neurologist? Neurology 1987;37(5):824–825. DOI: 10.1212/wnl.37.5.824.
63. Bin Rashid A, Kausik AK. AI revolutionizing industries worldwide: A comprehensive overview of its diverse applications. Hybrid Adv 2024;7:100277. DOI: 10.1016/j.hybadv.2024.100277.
64. Kiester L, Turp C. Artificial intelligence behind the scenes: PubMed's Best Match algorithm. J Med Libr Assoc 2022;110(1):15–22. DOI: 10.5195/jmla.2022.1236.
65. Hinostroza Fuentes VG, Karim HA, Tan MJT, et al. AI with agency: A vision for adaptive, efficient, and ethical healthcare. Front Digit Health 2025;7:1600216. DOI: 10.3389/fdgth.2025.1600216.

ORIGINAL RESEARCH

Clinical and Laboratory Features of Neonatal Seizures in Belarus

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Received on: 20 January 2026; *Accepted on*: 03 March 2026; *Published on*: 08 April 2026

Abstract

Introduction: Seizures are the most frequently-seen neurological emergency in neonates. The first weeks of life are a time of heightened risk for seizures due to age-dependent risk factors. Neonatal seizures are harmful to the developing brain; early and accurate diagnosis is critical.

Methods: We reviewed the clinical course of 75 infants serially admitted for convulsive seizures during the period 2016–2021 in the Grodno region of Belarus. All information relevant to the course of pregnancy and childbirth, gestational age, characteristics of seizures and data from laboratory and instrumental research methods were recorded.

Results: Most patients in our cohort were full-term (52/75, 69.3%). There was a history of severe obstetric complications in 69/75 (92%). A risk of miscarriage and acute respiratory viral infection had been recorded in the mother in 25/75 (33%) each. The main causative factors for seizures were hypoxic-ischemic encephalopathy (64/75, 85.3%), perinatal infections (52/75, 70%), metabolic disorders (24/75, 32%), and intracranial hemorrhages (20/75, 26.7%). Seizures were seen most often in the early neonatal period (61/75, 81.3%; $p < 0.001$). Motor automatisms (36/75, 48%) and clonic convulsions (30/75, 40%) were seen frequently.

Conclusion: Our team was able to identify motor seizures more frequently. The main causes of seizures were hypoxic-ischemic encephalopathy, perinatal infections, metabolic disorders, and intracranial hemorrhages but the etiology could not be identified in many cases. Further work is needed for timely identification of cause as etiology is a leading predictor of outcome.

Keywords: Clonic convulsions, Focal cerebral ischemia, Fosphenytoin, Grodno, Hypoxia, Hypoxic-ischaemic encephalopathy, Leukomalacia, Motor automatisms, Neonatal seizures, Neurological emergency, Neuronal excitation, Phenobarbital, Tandem mass spectrometry.

Newborn (2026): 10.5005/jp-journals-11002-0154

Key Points

- Seizures are the most frequently-seen neurological emergency in neonates.
- We reviewed the clinical course of 75 infants who were admitted continuously with convulsive seizures during the period 2016–2021 in the Grodno region of Belarus.
- Maternal history of severe obstetric complications and acute respiratory viral infection had been recorded in nearly a third of all patients. Most infants were born at full term and developed seizures in the early neonatal period.
- In our cohort, most patients had early-onset seizures caused by hypoxic-ischemic encephalopathy, perinatal infections, metabolic disorders, and intracranial hemorrhages. The etiology could not be identified in many. Further work is needed for a timely identification of cause because the etiology is a leading predictor of outcome.

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How to cite this article: Pratasevich TS, Yankouskaya N, Dzianisik N, *et al*. Clinical and Laboratory Features of Neonatal Seizures in Belarus. Newborn 2026;5(1):6–12.

Source of support: Nil

Conflict of interest: None

Background

Seizures are the most common neurological emergency in neonates, and this age group also has the highest incidence of seizures compared with any other period of life. The first weeks of life are a time of heightened risk for seizures due to physiological features of the developing brain that lead to increased neuronal excitation and decreased inhibition. Usually, seizures in neonates are a symptom of an acute brain injury; seizures are only rarely due to neonatal-onset epilepsy syndromes.

Neonatal seizures are harmful to the developing brain; early and accurate diagnosis is critical.[1–3] Seizures occur in 1–5 per 1,000 live births; incidence increases in preterm infants and in low-birth-weight infants. Seizures in the neonatal period differ considerably from those observed later in life with respect to their etiological profile and clinical presentation. In addition, the etiological profile in preterm infants is different from that seen in term infants. Hypoxic-ischemic encephalopathy is the most frequent cause of neonatal seizures in term babies followed by focal ischemia (stroke), cerebral malformations and metabolic disturbances.[4–7] A maternal infection in the prenatal period, congenital infections, diabetes, hypoglycemia, and a history of difficult birth are key indicators of potential perinatal asphyxia. The characteristics of other individuals

in the family should be examined for familial epileptic syndromes. Differences in etiology have been observed according to the level of national development.[4] Biochemical abnormalities occur either as an underlying cause or as an associated abnormality. Therefore, biochemical anomalies should be excluded in every case of neonatal seizure, despite the presence of other causes of neonatal seizures. In preterm neonates, intraventricular hemorrhage (IVH) and infections cause most of the seizures reported in this group. Better, prompt clinical and neuroimaging evaluations have reduced the number of undiagnosed cases, and the institution of newer neuroprotective strategies has improved the outcomes.[4,5,8]

Early recognition and treatment of these biochemical disturbances are essential for optimal management and satisfactory outcomes.[9] In this study, we examined the clinical course of 75 infants with convulsive seizures, admitted serially, who had complete records during the period 2016–2021 in the Grodno region of Belarus. Data relevant to the course of pregnancy and childbirth, gestational age, characteristics of seizures and data from laboratory and instrumental research methods were recorded. We present our findings in this brief report.

Methods

This study was designed as a retrospective review of risk factors for the development of convulsive syndrome in newborns in the Grodno region, as well as to analyze clinical characteristics and the results of laboratory and instrumental diagnostic tests. The total population of this region is estimated to be about 350,000. The infants were selected based on availability of complete records; five cases had to be excluded due to incomplete records. The study was approved by the institutional review board.

The following objectives were set for this study:

- To analyze complaints and leading clinical symptoms of seizure syndrome in children in Grodno and the Grodno region for the period 2016–2021.
- To assess the characteristics of the mothers' pregnancy and childbirth.
- To determine the most common causes of seizure syndrome in newborns. A total of 75 patients with neonatal seizures were included in the study for the period 2016–2021.

Clinical Evaluation

Special attention was paid to the course of pregnancy and childbirth, gestational age, characteristics of seizures, and data from laboratory and instrumental research methods. In the infants, the severity of organ-specific dysfunction was quantified as follows:

Encephalopathy

The diagnosis of neonatal encephalopathy was based on the main and additional criteria:[10]

Main Criteria

The presence of one of the following signs in the early neonatal (adaptation) period:

- Apgar score <5 at 5 minutes after birth;
- Need for resuscitation after birth or in the first 72 hours after birth;
- The presence of stable or progressive hypertensive-hydrocephalic syndrome;
- Impaired sucking and/or swallowing;
- Recurrent seizures;
- Coma;
- Organic changes revealed by cranial ultrasound, X-ray (if trauma is suspected), and magnetic resonance imaging (MRI) of the brain.

Presence of several (≥2–3) signs after the early neonatal adaptation:

- Absence or minimal expression of the concentration response;
- Central nervous system (CNS) arousal or depression;
- Minimal facial expressions, monotonous and unmodulated voice;
- Marked reduction or absence of the following reflexes: defensive, Babkin, support and automatic gait, Bauer crawl, upper and/or lower grasping;
- Spontaneous myoclonus.

Additional Criteria (risk factors for damage to the nervous system)

- Adverse medical history: A complicated obstetric history, chronic maternal illnesses, occupational exposure, and the age of the parents, especially the mother (under 18 or over 35 for primiparous women).
- Abnormal pregnancy: Threatened miscarriage, medication use and various medical interventions, acute maternal illnesses, etc.
- Abnormal labor: Prolonged or rapid, use of obstetric assistance or surgical interventions, Apgar score within 6–7 points.

Additional risk factors can be used in the diagnosis of neonatal encephalopathy in cases where neurological disorders are minimal and are on the borderline between normal and pathological.

Respiratory Distress

Respiratory failure in newborns is graded by clinical severity, typically mild (<3), moderate (4–6), and severe (>7) using the Silverman-Anderson or Downes' scores.[11] Increased work of breathing is associated with tachypnea, cyanosis, and grunting.

Post-hypoxic Cardiac Dysfunction

Inclusion criteria included a history of perinatal hypoxic-ischemic damage to the CNS such as cerebral ischemia and post-hypoxic cardiovascular disorders of the perinatal period [International classification of diseases (ICD)-X code P29], based on objective and/or instrumental examination data. Data from the cardiovascular system, such as impaired repolarization, altered cardiac rhythms and conduction disturbances on electrocardiograms, and impaired myocardial contractility with cardiac dilatation on echocardiography on postnatal days 4–6, was noted. In our intensive care units, we classify cardiac dysfunction using established grading systems.[12,13] In the respiratory system, based on gestational age, tachypnea, retractions, coughing, oliguria, lethargy, feeding difficulties, and insufficient weight gain may be observed. Some term infants develop signs of cardiorespiratory failure with hepatomegaly and/or splenomegaly.

Other Information

We also add the following information to our statistical models:

- ICD-10 code for unspecified perinatal infections;
- Signs of systemic inflammatory response syndrome. This code is when a newborn exhibits signs of inflammation without an identified pathogen or clear localization.

- ICD-10 code P36.3 for sepsis in the newborn due to other and unspecified staphylococci
  - P36.8 sepsis in the newborn due to other bacterial agents
  - P36.2 sepsis in the newborn due to *Staphylococcus aureus*

Laboratory Findings

To confirm infection as a possible cause, a blood count and acute phase reactants were examined, and a lumbar puncture was performed to evaluate the cerebrospinal fluid (CSF). Blood gas profiles, serum lactate and amino acids, urine organic acids, and a tandem acylcarnitine profile were examined if a metabolic disorder was suspected. Magnetic resonance imaging is accepted as the gold standard imaging modality.

In our laboratory, normal total white blood cell (WBC) counts are 14 (9.6–17.8) $\times 10^9$/L. The range of segmented neutrophils was 51 (34–63)%, band neutrophils 4 (2–6)%, lymphocytes 31 (20–43)%, monocytes 9 (7–11)%, eosinophils 2 (1–4)%, and basophils 1 (1–1)%. An immature-to-total neutrophil (I:T) count > 0.2 is noted.

We define normal serum sodium levels as 132–146 mmol/L and hypocalcemia as <2 mmol/L in full-term and <1.75 mmol/L in preterm infants. Procalcitonin concentrations (enzyme immunoassay) of 0.1–0.5 ng/mL may indicate a low risk of a clinically-significant bacterial infection requiring antibiotic treatment, 0.5–2 a low risk of sepsis or septic shock, and >2 ng/mL indicates a risk of severe sepsis or septic shock.

Tandem mass spectrometry is usually performed for neurological disorders with disturbances of consciousness (lethargy, coma), focal/generalized seizures, changes in muscle tone, opercular automatisms, pedaling movements of the lower extremities, etc. Hereditary disorders of amino acid and organic acid metabolism and mitochondrial β-oxidation defects are the largest groups of hereditary disorders (more than 100 nosological entities). Most of these diseases manifest in early childhood and are characterized by an acute onset with neurological manifestations.

Reference values for leukocytes in CSF analysis: WBC counts of 0–30 $\times 10^6$/L; 5–35% lymphocytes, 50–90% monocytes, and 0–8% neutrophils.

Imaging

In our protocols, we perform CNS imaging to diagnose hypoxic-ischemic changes in the brain when the following changes are seen:

Cranial Sonography

In full-term newborns:

- Parasagittal ischemic necrosis
- Ischemic lesions of the basal ganglia and thalamus
- Subcortical leukomalacia
- Diffuse hypoxic-ischemic changes in the acute period
- Cerebral edema
- Multicystic encephalomalacia (total cystic degeneration)
- Focal ischemic brain lesions.

In premature infants:

- Periventricular leukomalacia (PVL)
- Diffuse leukomalacia (PVL + subcortical leukomalacia)
- Periventricular hemorrhagic infarction.

Computed tomography (CT)/magnetic resonance imaging (MRI) of the brain is performed based on clinical decisions, considering the patient's severity of illness and the possibility of structural pathology of the brain, cerebral hemorrhages, and malformations of the cerebral cortex.

Table 1: Clinically-relevant findings in pre- and perinatal records

Findings	*Number*
Delivery	
Vaginal	44 (58.7%)
Cesarean section	31 (41.3%)
Primigravida	31 (41.3%)
Second pregnancy	17 (22.7%)
Third or later pregnancy	27 (36%)
Prior complicated obstetric history	69 (92%)***
Antepartum records	2 (2.7%)
Acute respiratory viral infections	28 (37.3%)
Threatened miscarriage	27 (36%)
Anemia	13 (17.3%)
Findings suggestive of acute fetal hypoxia during delivery	12 (16%)
Prolonged (>18 hours) premature rupture of membranes (<36 weeks' gestation); PPROM with an anhydrous period >12 hours	8 (10.7%)
Weak labor	6 (8%)
Umbilical cord prolapse	2 (2.7%)
Umbilical cord entanglement	15 (20%)

**$p < 0.01$, ***$p < 0.001$. PPROM, preterm prelabor rupture of membranes

Treatment

We use diazepam as a first-line drug for emergency care. However, a number of limitations often necessitate the selection and administration of alternative therapies for both controlling the seizures and providing maintenance therapy. Phenobarbital monotherapy is effective in no more than half of all infants with seizures.

Statistical Analysis

Data were processed using traditional methods of variation statistics with the application software packages "STATISTICA 10.0" and "EXCEL". Non-parametric data were shown as percentages or as median/interquartile ranges and analyzed using the Mann–Whitney/Kruskal-Wallis tests.[14–16] When comparing groups with a limited number of observations, the two-tailed Fisher test was used.[16,17]

Results

Obstetric records showed that 44 (58.7%) infants were born vaginally, and 31 (41.3%) were born by cesarean section. Thirty-one (41.3%) mothers were primigravidae; 17 (22.7%) had this as a second pregnancy, and 27 (36%) were in their third and subsequent pregnancies. Findings suggestive of a complicated maternal clinical course are listed in Table 1.

In our cohort, 52/75 (69.3%) infants were born at full-term at a gestational age (median, range) of 39^{+2} (39–40) weeks. Preterm infants were born at 35 (32^{+1}–36^{+6}) weeks, $p < 0.0001$. The clinical features are summarized in Table 2.

Table 2: Clinical findings of infants with seizures

Findings	*Number*
Gestational age (median, IQR) weeks; 75 infants	37.2 (33^{+3}–39) weeks
Term; 52 infants (69.3%)	39^{+2} (39–40) weeks
Preterm; 23 infants (30.7%)	35 (32^{+1}–36^{+6}) weeks***
Birth weight (median, IQR)	3150 (2700–3800) grams
Length (median, IQR)	51 (49–54) cm
Gender	
Males	43 (57.3%)
Females	32 (42.7%); difference not significant
Birth asphyxia	21 (28%)
Multisystem support with assisted ventilation	49 (65.3%)**
Neonatal encephalopathy	64 (85.3%)***
Culture-positive invasive perinatal infections	9/75 (12%)***
Blood	3 (4%)
CSF	2 (2.7%)
Endotracheal tube	4 (5.3%)
Respiratory failure	50 (66.7%)***
Grade I	6 (8%)
Grade II	18 (2%)
Grade III	26 (34.7%)***
Cardiac dysfunction	16 (21.3%)

**$p < 0.01$, ***$p < 0.001$. IQR, interquartile range; CSF, cerebrospinal fluid

Table 3: Seizures

Findings	*Number*
Types of seizures	
Clonic contractions	30 (40%)
Tonic spasms	21 (28%); neck hyperextension in 11
Myoclonus in hypernatremia	20 (26.7%)
Subtle seizures	
Convulsions of diaphragm	18 (24%)
Motor automatisms	36 (48%)
Oral automatisms	17 (22.7%)
Rowing arm movements, pedaling with legs	12 (16%)
Ocular (such as nystagmus)	7 (9.3%)
Atypical movements	12 (16%)
Apnea with desaturation	8 (10.7%)
Vegetative movements	4 (5.3%)
Mydriasis	2 (2.7%)
Transient skin redness	1 (1.3%)
Gagging	1 (1.3%)

Most seizures occurred within the first 48 hours after birth (<48 hours: 46 (61.3%; postnatal days 3–7: 15 (20%); and >7 days: 14 (18.7%; $p = 0.00001$). Table 3 lists the types of seizures.

Table 4: Laboratory findings

Findings	*Number*
Altered WBC counts	
Leukocytosis	25 (33.3%)
Leukopenia	10 (13.3%)
Bandemia with I:T >0.2	39 (52%)
Biochemical abnormalities	24 (32%)
Serum Na range (median, IQR)	128.4 (124.1–129.3) mmol/L
Hyponatremia (median, IQR)	6 (8%); 128.4 (124.1–129.3) mmol/L
Hypernatremia	1 (151.1 mmol/L)
Serum Ca range (median, IQR)	1.8 (1.5–1.9) mmol/L
Hypocalcemia	16 (21.3%)
Hypoglycemia (blood glucose ≤1.5 mmol/L	1 (0.13%)
Increased C-reactive protein	27 (36%)
Increased procalcitonin	6 (8%). Measured 6.43 (0.82–37.3) ng/mL

IQR, interquartile range; I:T, immature:total neutrophil counts

Table 5: Findings on cranial ultrasonography

Findings	*Number*
Intracranial hemorrhages	20 (26.7%)
Intraventricular hemorrhage	8 (10.7%)
Grade II	3 (4%)
Grade III	5 (6.7%)
Epidural hemorrhage	2 (2.7%)
Subdural hemorrhage	2 (2.7%)
Subarachnoid hemorrhage	6 (8%)
Epidural hemorrhage	2 (2.7%)
Intracerebral hemorrhage in 2	2 (2.7%)
Ventricular dilatation	36 (48%)
Hypoxic-ischemic changes	17 (22.7%)

Laboratory findings are summarized in Table 4. Tandem mass spectrometry data showed decreased free carnitine levels in 1 (1.3%) infant. Cerebrospinal fluid showed neutrophilic pleocytosis in 20 (26.7%) newborns, and increased red blood cells (hemorrhage) in 25 (33.3%).

Microbiology records showed positive cultures from invasive sites in 9/75 (12%): endotracheal tube [4 (5.3%)], blood [3 (4%)], and CSF [2 (2.7%)]. Most infections involved Gram-positive bacteria: Staphylococci [48% (*Staphylococcus haemolyticus, Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus lentus, and Staphylococcus warneri*)] and Streptococci (9.3%). A few showed Gram-negative bacteria [*Klebsiella pneumoniae* (8%), *Escherichia faecium* (8%), and *Escherichia coli* (2.7%)].

In our protocols, we routinely perform CNS imaging to evaluate encephalopathic changes. Cranial ultrasonography was performed on all 75 infants. The findings are shown in Table 5.

Magnetic resonance imaging was performed in 31 (41.3%). The findings are shown in Table 6.

Table 6: Findings on MRI. Percentages are expressed in the cohort of 75

Findings	*Number*
Cystic-glial transformation	6 (8%)
Post-ischemic leukomalacia (periventricular leukomalacia)	4 (5.3%)
Subcortical dysmetabolic changes (T2-hyperintense signals, white matter lesions, or volumetric reductions in thalamus and caudate nucleus)	2 (2.7%)
Congenital defects	8 (10.7%)
Dandy-Walker malformation type 1	2 (2.7%)
Deviated internal carotid artery	2 (2.7%)
Hypoplasia of the septum pellucidum	1 (1.3%)
Aneurysm of the lateral villous artery	1 (1.3%)
Hypoplasia of the cerebellar hemispheres	1 (1.3%)
Dural arteriovenous fistula	1 (1.3%)

Table 7: Findings on EEG

Findings	*Number*
Indirect signs of immaturity of brain structures	3 (4%)
Severe disorganization of the bioelectrical activity of the brain	2 (2.7%)
Diffuse changes in cortical rhythms	1 (1.3%)
Flat EEG	4 (5.3%)
No abnormalities	9 (12%)

EEG, electroencephalography

We use electroencephalography (EEG) for monitoring infants with encephalopathy.[2,18–20] In this cohort, 19 (25.3%) patients were monitored (Table 7).

To treat seizures, we use intravenous diazepam as the first-line drug. If necessary, phenobarbital is then prescribed for maintenance therapy. In the analyzed group, diazepam was used in 56 (74.7%) newborns, phenobarbital in 48 (64%), and Depakine in 11 (14.7%). Sodium oxybutyrate was used in 8 (10.7%). Eighteen (24%) infants continued to have seizures after diazepam. In 30 (49%), a recurrence was observed in within 24 hours after stopping diazepam. Seizures stopped spontaneously in 8 (10.7%) infants. Maintenance therapy with phenobarbital was carried out at a dose of 5 mg/kg/day. In 11 cases, due to phenobarbital's ineffectiveness, Depakine was prescribed.

Discussion

The first weeks of life represent a period of heightened vulnerability to seizures because of age-dependent physiological characteristics of the developing brain.[21,22] During the neonatal period, neuronal networks are intrinsically more excitable due to an increased density and activity of excitatory glutamatergic receptors and an immature inhibitory γ-aminobutyric acid (GABA) system.[23,24] During early development, GABA can exert depolarizing (excitatory) rather than inhibitory effects because of elevated intracellular chloride levels, further promoting neuronal excitation.[25] Additionally, incomplete myelination, enhanced synaptic plasticity, and evolving cortical connectivity contribute to an imbalance between excitation and inhibition.[26,27] Together, these factors create a neurobiological environment in which seizures are more easily initiated and propagated during the first weeks of life.[28]

Neonatal health in Belarus is considered strong by global standards, with one of the lowest neonatal mortality rates in Eastern Europe.[29] Over the past three decades, the country has achieved a substantial decline in deaths within the first 28 days of life, largely due to universal access to facility-based deliveries, a well-structured three-tier perinatal care system, and high coverage of skilled birth attendants.[30] Comprehensive prenatal screening programs, early ultrasound assessments, and effective referral networks for high-risk pregnancies have further improved outcomes. Advances in neonatal intensive care have increased survival among preterm and very low birth weight infants. Despite demographic challenges such as declining birth rates, the overall organization of maternal and newborn healthcare services continues to support favorable neonatal outcomes nationwide.

As in other parts of the world, neonatal seizures are defined in Belarus as sudden, abnormal electrical discharges in the brain occurring in the first 28 days of life.[31] These are recognized as an important acute neurological condition in newborn care and are covered in national clinical guidance for neonatal medicine. A clinical protocol from the Republican Centre for Health Development (Ministry of Health of Belarus) lists "seizures of the newborn" (ICD-10 code P90) as a specific neonatal condition requiring structured diagnosis and management within perinatal and neonatal services, with standardized approaches to evaluation and treatment outlined at different levels of health care.[32] We are still compiling precise Belarus-specific prevalence data.[33] International pediatric neurology literature indicates that neonatal seizures occur in roughly 1–5 per 1,000 term infants and significantly more often among preterm babies. Major causes include hypoxic-ischemic encephalopathy (HIE), intracranial hemorrhage, infections, and metabolic disturbances associated with acute CNS dysfunction.[4–7,9]

Seizures due to an intracranial process vs. those due to systemic disorders cannot always be reliably distinguished by clinical manifestations (such as focal or generalized seizures).[34] Neuroimaging methods can help in investigating these cases.[35,36] Transfontanelle ultrasonography, v-cEEG, CT, and MRI can be helpful.[37] In experienced hands, ultrasound is an outstanding tool to detect brain abnormalities in preterm and full-term infants, to follow the progression of these lesions, and to describe the maturation of the infant brain.[18] Video-continuous EEG has also been shown to be useful and should be initiated as soon as is feasible, in order to evaluate for events of concern, screen for subclinical seizures, and assess the EEG background.[16] The goal is to confirm diagnosis, identify etiology, and guide treatment. These can manifest with subtle or focal movements that are difficult to distinguish from other neonatal behaviors, making EEG monitoring particularly important.[38] The management and outcomes vary with the underlying cause, gestational age, and severity; some seizures resolve with treatment of the provoking condition, whereas others may portend longer-term neurological sequelae such as epilepsy or developmental impairment, especially in preterm infants or those with severe brain injury.[39,40]

In our study, 92% of pregnant women had a complicated obstetric history; one-third had an acute respiratory viral infection and another third a threatened miscarriage. Nearly 60% experienced episodes of intrapartum hypoxia. Factors associated with neonatal seizures were HIE (85.3%), infections (69.3%), metabolic disorders (32%), and intracranial hemorrhages (26.7%). Seizures were seen most often in the early neonatal period (81.3%). Even though the cohort was selected based on clinically-obvious seizures, nearly

half also showed subtle signs. Acute symptomatic seizures in many newborns can be caused by yet unidentified factors, calling for further investigation.

Neonatal sepsis was not a leading cause of neurological morbidity in our series. Most of the isolates were Gram-positive bacteria, and the spectrum differed from those in other countries. Staphylococci appeared most frequently, as noted in previous reports from Eastern Europe.[41] Group B streptococci were not observed as frequently. *Staphylococcus haemolyticus* is a coagulase-negative staphylococcus (CoNS) - a Gram-positive, catalase-positive bacterium that is part of the normal skin flora but acts as an opportunistic pathogen, commonly associated with hospital-acquired infections and biofilm formation on medical devices.[42] It is known for high levels of antibiotic resistance.[42] Similar to other parts of the world, we also need protocols to reduce variability in maternal screening, infection control measures, and antimicrobial stewardship programs.[43] Strengthening perinatal prevention strategies and neonatal intensive care unit (NICU) infection control will help in reducing disease burden.[44]

Seizures are common in neonates, but there is considerable variability in clinical management. According to the literature, phenobarbital is used as the first-line antiseizure medication regardless of etiology.[4,7,45,46] It is seen to be more effective than levetiracetam and easier to administer than fosphenytoin, and there are some data suggest it might even be safe to discontinue the phenobarbital after the acute seizures resolve, prior to hospital discharge.[6] However, in our experience, phenobarbital monotherapy is effective in only about half of all infants with seizures. Identification and correction of transient metabolic disturbances improve outcomes, but about a third of all patients still require combination therapy.[47] A standardized pathway is still needed for clinical management. We have also developed educational materials to inform parents and guardians about the diagnosis and initial treatment options.[45]

Prognosis depends on the etiology: long-term outcomes of neonatal seizures are variable, ranging from no neurologic abnormalities to developmental delay, intellectual disability, and epilepsy.[4] Most neonates with seizures due to a transient electrolyte disturbance, such as hypocalcemia and hyponatremia, do well when seizures resolve after the disturbance is reversed and long-term antiseizure medications are not required.[28] However, those with severe intraventricular hemorrhage show high morbidity and mortality.[48] For idiopathic seizures or seizures due to brain malformations, earlier onset is associated with worse neurodevelopmental outcomes.[39] The outcomes of newborns impacted by non-metabolic neonatal seizures can improve with comprehensive clinical and laboratory evaluation and appropriate therapy.[9]

Similar to other parts of the world, neonatal seizures are viewed as a neurological emergency in Belarus. These call for prompt recognition and structured management. We need standardized clinical protocols and access to specialized neonatal services to support early diagnosis, appropriate treatment, and timely referral of high-risk infants. As survival of preterm and critically-ill newborns continues to improve, ongoing emphasis on neurocritical monitoring, timely etiologic evaluation, and long-term neurodevelopmental follow-up will remain essential to minimize adverse outcomes and further strengthen neonatal neurological care in Belarus.[49]

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References

1. Shellhaas RA. Seizure classification, etiology, and management. Handb Clin Neurol 2019;162:347–361. DOI: 10.1016/B978-0-444-64029-1.00017-5.
2. Orman G, Benson JE, Kweldam CF, et al. Neonatal head ultrasonography today: A powerful imaging tool! J Neuroimaging 2015;25(1):31–55. DOI: 10.1111/jon.12108.
3. WHO. Guidelines on neonatal seizures. Geneva, Switzerland: World Health Organization; 2011. Available from: https://www.who.int/publications/i/item/9789241548304. [Last accessed on: 01 January 2011].
4. Acar DB, Bulbul A, Uslu S. Current overview of neonatal convulsions. Sisli Etfal Hastan Tip Bul 2019;53(1):1–6. DOI: 10.14744/SEMB.2018.22844.
5. Vasudevan C, Levene M. Epidemiology and aetiology of neonatal seizures. Semin Fetal Neonatal Med 2013;18(4):185–191. DOI: 10.1016/j.siny.2013.05.008.
6. DeLaGarza-Pineda O, Mailo JA, Boylan G, et al. Management of seizures in neonates with neonatal encephalopathy treated with hypothermia. Semin Fetal Neonatal Med 2021;26(4):101279. DOI: 10.1016/j.siny.2021.101279.
7. Anwar T, Triplett RL, Ahmed A, et al. Treating seizures and improving newborn outcomes for infants with hypoxic-ischemic encephalopathy. Clin Perinatol 2024;51(3):573–586. DOI: 10.1016/j.clp.2024.04.013.
8. Sandoval Karamian AG, DiGiovine MP, Massey SL. Neonatal seizures. Pediatr Rev 2024;45(7):381–393. DOI: 10.1542/pir.2023-006016.
9. Rajesh Reddy K, Potpalle D, Mohammed AM, et al. Neonatal non-metabolic seizure distribution in relation to biochemical abnormalities. Cureus 2025;17(2):e79723. DOI: 10.7759/cureus.79723.
10. Shanko GG, Shishko GA, Ulezko EA. Clinical diagnostics of encephalopathy in newborns and birth-related trauma. Minsk, Belarus: Belarusian Medical Academy of Postgraduate Education, Research Institute of Maternal and Child Health; 2003. pp. 1–100.
11. Mathai SS, Raju U, Kanitkar M. Management of respiratory distress in the newborn. Med J Armed Forces India 2007;63(3):269–272. DOI: 10.1016/S0377-1237(07)80152-3.
12. Hassan ES, Ahmad S-EA, Mohamad IL, et al. The value of modified Ross score in the evaluation of children with severe lower respiratory tract infection admitted to the pediatric intensive care unit. Eur J Pediatr 2023;182(2):741–747. DOI: 10.1007/s00431-022-04737-9.
13. Jayaprasad N. Heart failure in children. Heart Views 2016;17(3):92–99. DOI: 10.4103/1995-705X.192556.
14. Hart A. Mann-Whitney test is not just a test of medians: Differences in spread can be important. BMJ 2001;323(7309):391–393. DOI: 10.1136/bmj.323.7309.391.
15. Habibzadeh F. Data distribution: Normal or abnormal? J Korean Med Sci 2024;39(3):e35. DOI: 10.3346/jkms.2024.39.e35.
16. Abend NS, Wusthoff CJ. Neonatal seizures and status epilepticus. J Clin Neurophysiol 2012;29(5):441–448. DOI: 10.1097/WNP.0b013e31826bd90d.
17. Balasubramaniam M, Fischgrund JS. Two-tailed Fisher's exact test (Cochran-Mantel-Haenzel). Spine J 2002;2(2):158–159. DOI: 10.1016/s1529-9430(02)00143-2.
18. Gettings JV, Soul JS. Updates in neonatal seizures. Clin Perinatol 2025;52(2):375–393. DOI: 10.1016/j.clp.2025.02.008.
19. Samanta D. Recent advances in the diagnosis and treatment of neonatal seizures. Neuropediatrics 2021;52(2):73–83. DOI: 10.1055/s-0040-1721702.
20. Ryan MAJ, Malhotra A. Electrographic monitoring for seizure detection in the neonatal unit: Current status and future direction. Pediatr Res 2024;96(4):896–904. DOI: 10.1038/s41390-024-03207-2.

21. Holmes GL, Khazipov R, Ben-Ari Y. New concepts in neonatal seizures. Neuroreport 2002;13(1):A3–A8. DOI: 10.1097/00001756-200201210-00002.
22. Moshé SL. Seizures in the developing brain. Neurology 1993;43(11 Suppl 5):S3–S7. PMID: 8232986.
23. Cherubini E, Gaiarsa JL, Ben-Ari Y. GABA: An excitatory transmitter in early postnatal life. Trends Neurosci 1991;14(12):515–519. DOI: 10.1016/0166-2236(91)90003-d.
24. Basu SK, Pradhan S, du Plessis AJ, et al. GABA and glutamate in the preterm neonatal brain: In-vivo measurement by magnetic resonance spectroscopy. Neuroimage 2021;238:118215. DOI: 10.1016/j.neuroimage.2021.118215.
25. Watanabe M, Fukuda A. Development and regulation of chloride homeostasis in the central nervous system. Front Cell Neurosci 2015;9:371. DOI: 10.3389/fncel.2015.00371.
26. Fotiadis P, Cieslak M, He X, et al. Myelination and excitation-inhibition balance synergistically shape structure-function coupling across the human cortex. Nat Commun 2023;14(1):6115. DOI: 10.1038/s41467-023-41686-9.
27. Kratimenos P, Sanidas G, Simonti G, et al. The shifting landscape of the preterm brain. Neuron 2025;113(13):2042–2064. DOI: 10.1016/j.neuron.2025.03.024.
28. Jensen FE. Developmental factors in the pathogenesis of neonatal seizures. J Pediatr Neurol 2009;7(1):5–12. DOI: 10.3233/JPN-2009-0270.
29. Maugeri A, Barchitta M, Schillaci G, et al. Spatial patterns and temporal trends in stillbirth, neonatal, and infant mortality: An exploration of country-level data from 2000 to 2021. J Glob Health 2025;15:04034. DOI: 10.7189/jogh.15.04034.
30. Network A. Maternal health across borders: Lessons from Belarus and Azerbaijan's diverging approaches Warsaw, Poland: Foundation for women and family planning; 2024. Available from: https://astra.org.pl/maternal-health-across-borders-learning-from-belarus-and-azerbaijans-approaches/. [Last accessed on: 28 October 2024].
31. Panayiotopoulos CP. Chapter 5, Neonatal seizures and neonatal syndromes. In: Panayiotopoulos CP, editor. The Epilepsies: Seizures, Syndromes and Management. 1. Oxfordshire, United Kingdom: Bladon Medical Publishing; 2005. pp. 1–99.
32. UNDP. Health comes first: Belarus Puts healthcare at the heart of its sustainable development agenda Minsk, Belarus: United Nations Development Programme Office in Belarus; 2025. Available from: https://www.undp.org/belarus/stories/health-comes-first-belarus-puts-healthcare-heart-its-sustainable-development-agenda. [Last accessed on: 18 April 2025].
33. Shalkevich LV, Kudlatch AI, Volchok VI. The epidemiology of epilepsy in children in the Republic of Belarus. Zh Nevrol Psikhiatr Im S S Korsakova 2016;116(11):98–102. DOI: 10.17116/jnevro201611611198-102.
34. Soul JS. Acute symptomatic seizures in term neonates: Etiologies and treatments. Semin Fetal Neonatal Med 2018;23(3):183–190. DOI: 10.1016/j.siny.2018.02.002.
35. Weeke LC, Van Rooij LG, Toet MC, et al. Neuroimaging in neonatal seizures. Epileptic Disord 2015;17(1):1–11. DOI: 10.1684/epd.2015.0730.
36. Pisani F, Spagnoli C, Falsaperla R, et al. Seizures in the neonate: A review of etiologies and outcomes. Seizure 2021;85:48–56. DOI: 10.1016/j.seizure.2020.12.023.
37. Mussavi M, Khanzadeh S, Sadeghvand S, et al. Brain CT scan, sonography and EEG findings in neonates with seizure. Iran J Child Neurol 2022;16(3):183–191. DOI: 10.22037/ijcn.v17i1.34950.
38. Kim EH, Shin J, Lee BK. Neonatal seizures: Diagnostic updates based on new definition and classification. Clin Exp Pediatr 2022;65(8):387–397. DOI: 10.3345/cep.2021.01361.
39. Kang SK, Kadam SD. Neonatal seizures: Impact on neurodevelopmental outcomes. Front Pediatr 2015;3:101. DOI: 10.3389/fped.2015.00101.
40. Westergren H, Finder M, Marell-Hesla H, et al. Neurological outcomes and mortality after neonatal seizures with electroencephalographical verification. A systematic review. Eur J Paediatr Neurol 2024;49:45–54. DOI: 10.1016/j.ejpn.2024.02.005.
41. Kornienko M, Ilina E, Lubasovskaya L, et al. Analysis of nosocomial Staphylococcus haemolyticus by MLST and MALDI-TOF mass spectrometry. Infect Genet Evol 2016;39:99–105. DOI: 10.1016/j.meegid.2015.12.015.
42. Eltwisy HO, Twisy HO, Hafez MH, et al. Clinical infections, antibiotic resistance, and pathogenesis of Staphylococcus haemolyticus. Microorganisms 2022;10(6):1130. DOI: 10.3390/microorganisms10061130.
43. Mzumara GW, Mambiya M, Iroh Tam PY. Protocols, policies and practices for antimicrobial stewardship in hospitalized patients in least-developed and low-income countries: A systematic review. Antimicrob Resist Infect Control 2023;12(1):131. DOI: 10.1186/s13756-023-01335-8.
44. Johnson J, Akinboyo IC, Schaffzin JK. Infection prevention in the neonatal intensive care unit. Clin Perinatol 2021;48(2):413–429. DOI: 10.1016/j.clp.2021.03.011.
45. Pisani F, Pavlidis E. The role of electroencephalogram in neonatal seizure detection. Expert Rev Neurother 2018;18(2):95–100. DOI: 10.1080/14737175.2018.1413352.
46. Abiramalatha T, Thanigainathan S, Ramaswamy VV, et al. Anti-seizure medications for neonates with seizures. Cochrane Database Syst Rev 2023;10(10):CD014967. DOI: 10.1002/14651858.CD014967.pub2.
47. Lewis CB, Patel P, Adams N. Phenobarbital. Treasure Island, Florida, USA: StatPearls Publishing; 2025. Available from: https://www.ncbi.nlm.nih.gov/sites/books/NBK532277/. [Last accessed on 28 February 2024].
48. Heljic S, Uzicanin S, Catibusic F, et al. Predictors of mortality in neonates with seizures; a prospective cohort study. Med Arch 2016;70(3):182–185. DOI: 10.5455/medarh.2016.70.182-185.
49. Petersen MA, Glass HC. Extending the branches of neonatal neurocritical care. Semin Perinatol 2025;49(7):152131. DOI: 10.1016/j.semperi.2025.152131.

REVIEW ARTICLE

Meckel Diverticulum

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Received on: 12 January 2026; *Accepted on*: 24 February 2026; *Published on*: 08 April 2026

Abstract

Meckel diverticulum (MD) is a congenital pouch on the antimesenteric border of the distal ileum. It is a remnant of the omphalomesenteric (vitelline) duct. These anomalies are seen all over the world with a fairly consistent prevalence of 0.2–4%. There is no strong evidence for genetic factors. There can be seen three possible variants: (1) an MD with the rest of the omphalomesenteric duct (OMD) seen as a terminal fibrous band connecting with the umbilicus; (2) the whole OMD remains patent; and (3) an umbilical cyst, a fluid-filled remnant in a part of the ductal pathway. The cause is unknown; no single gene mutation has been consistently associated with MDs, but new information is emerging about the pathogenesis of gut heterotopias. Some of these diverticula are seen in patients with trisomy 13, trisomy 18, trisomy 21, or with broader patterns of multiple congenital anomalies, including the Vertebral, Anal, Cardiac, tracheoesophageal, Renal, Limb anomalies (VACTERL) association. Meckel diverticulums can be associated with fibrous peritoneal bands, which can be associated with mechanical intestinal obstruction by kinking, compressing, or entrapping small bowel loops. Symptomatic cases may be diagnosed with a nuclear medicine technetium-99m pertechnetate Meckel scan that detects gastric tissue. Asymptomatic patients may be closely followed with conservative management. However, for symptomatic cases, a diverticulectomy is performed, and the fibrous bands or adhesions are released. If the base is broad, a segmental ileal resection is considered.

Keywords: Antimesenteric border, Auerbach, Bone morphogenetic protein, Calprotectin, Congenital pouch, Diverticulectomy, Ectopic gastric tissue, Ectopic pancreatic tissue, Eosinophilia, Fibroblast growth factors, Fibrous peritoneal bands, Homeobox gene, Infant, Meckel scan, Meissner, Neonate, Newborn, Nod-like receptor family CARD domain containing 4, Nod-like receptor pathway, Omphalomesenteric duct, Segmental ileal resection, Sonic hedgehog pathway, Technetium-99m pertechnetate, Trisomy 13, Trisomy 18, Trisomy 21, Umbilical cyst, VACTERL association, Vitelline duct, Volvulus, Wnt/β-catenin signaling, Yolk sac.

Newborn (2026): 10.5005/jp-journals-11002-0149

Key Points

- Meckel diverticulum (MD) is a congenital pouch on the antimesenteric border of the distal ileum. It is a remnant of the omphalomesenteric (vitelline) duct.
- Meckel diverticulum is seen all over the world with a fairly consistent prevalence of 0.2–4%. There is no strong evidence for genetic factors.
- Meckel diverticulums can be seen in three possible variants: (1) an MD with the rest of the omphalomesenteric duct (OMD) seen as a terminal fibrous band connecting with the umbilicus; (2) the whole OMD remains patent; and (3) an umbilical cyst, a fluid-filled remnant in a part of the ductal pathway.
- No single gene mutation has been found to be consistently associated with MDs. Sometimes these diverticulae are seen in patients with trisomy 13, trisomy 18, trisomy 21, or with broader patterns of multiple congenital anomalies, including the Vertebral, Anal, Cardiac, tracheoesophageal, Renal, Limb anomalies (VACTERL) association.
- Meckel diverticulums can be associated with fibrous peritoneal bands, which can be associated with mechanical intestinal obstruction by kinking, compressing, or entrapping small bowel loops.
- Symptomatic cases may be diagnosed with a nuclear medicine technetium-99m pertechnetate Meckel scan that detects gastric tissue.
- Asymptomatic patients may be closely followed with conservative management. However, for symptomatic cases, a diverticulectomy is performed. If the base is broad, a segmental ileal resection is considered. Surgical release of fibrous bands or adhesions may also be necessary.

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How to cite this article: Khare AK. Meckel Diverticulum. Newborn 2026;5(1):13–19.

Source of support: Nil

Conflict of interest: None

Introduction

An MD is a congenital pouch on the antimesenteric border of the distal ileum. It is a remnant of the OMD (vitelline duct) that connects the developing embryo with the yolk sac, and its location corresponds to the terminal portion of the midgut.[1–3] The MD is frequently described using a rule of 2;[4] it is seen in 2% of the population, often presents before age 2, and may contain two types of ectopic tissue (gastric or pancreatic). In adults with a persisting MD, the diverticulum is about 2 inches long and is located 2 feet from the ileocecal valve. In many patients, these diverticula do not cause symptoms. However, others can present with peptic ulceration, bleeding, inflammation, gastrointestinal dysmotility, obstruction, volvulus, or intussusception.[2,3,5–8]

In this review, we present a synopsis of our current understanding of the pathophysiology, clinical features, diagnosis, and management of MD. We have combined some data from our own preliminary studies with an extensive literature search in Embase, PubMed, and Scopus. To avoid bias in identification of

studies, keywords were short-listed *a priori* from PubMed's Medical Subject Headings (MeSH) thesaurus.

Epidemiology

Meckel diverticula are seen all over the world with a fairly consistent prevalence of 0.2–4%.[1,3,6,9] There is no strong evidence for increased risk due to ethnic/racial factors, maternal nutrition, or environmental toxins. However, a few series have noted higher numbers in males with male-to-female ratios as high as 2:1.[10] Some hospital-based studies from Europe and North America have reported slightly higher frequencies than in the rest of the world, but these also might be related to study methods, access to imaging, rates of surgery, and autopsy rates, rather than to true genetic or environmental differences.[7,11]

Normal Gut Morphogenesis—Possible Insights into the Development of MDs

During development, the primitive gut continues to communicate with the yolk sac through the OMD.[12] Normal gut morphogenesis and apoptosis, which are controlled by multiple genes and signaling pathways, include:

- Homeobox (HOX) genes, which regulate anteroposterior patterning of the gut. Abnormal HOX expression can affect midgut differentiation and vitelline duct regression;[13,14]
- Sonic hedgehog (SHH) pathway is critical for intestinal epithelial-mesenchymal signaling and proper midgut development;[15,16]
- Fibroblast growth factors (FGFs) are involved in gut elongation and rotation; disturbances may interfere with duct involution;[17,18]
- Bone morphogenetic protein (BMP) signaling contributes to apoptosis and regression of transient embryonic structures, including the vitelline duct;[19,20] and
- Wnt/β-catenin signaling that regulates intestinal stem cell proliferation and differentiation to indirectly affect midgut remodeling.[15,21]

The pathogenesis of gastric heterotopia in MD is likely rooted in incomplete obliteration of the vitelline duct during embryonic development and misplaced gastric/pancreatic tissue within the diverticulum.[3,22] Meckel diverticulum results from the failure of involution of the OMD; these aberrations may result from abnormal apoptotic signaling, genetic defects, or mechanical factors that affect gut folding and positioning.[3,23]

Meckel diverticulums are true congenital diverticula composed of all layers of the intestinal wall and do not inherently have a tendency to become fibrotic.[3] These may show ectopic presence of embryonic cells that differentiate into gastric mucosa (50%), pancreatic cells (15%), and others, such as duodenal/colonic mucosa in others.[5] In some cases, fibrous vitelline or omphalomesenteric bands can persist as straps connecting the diverticulum to the umbilicus or abdominal wall.

Genetics

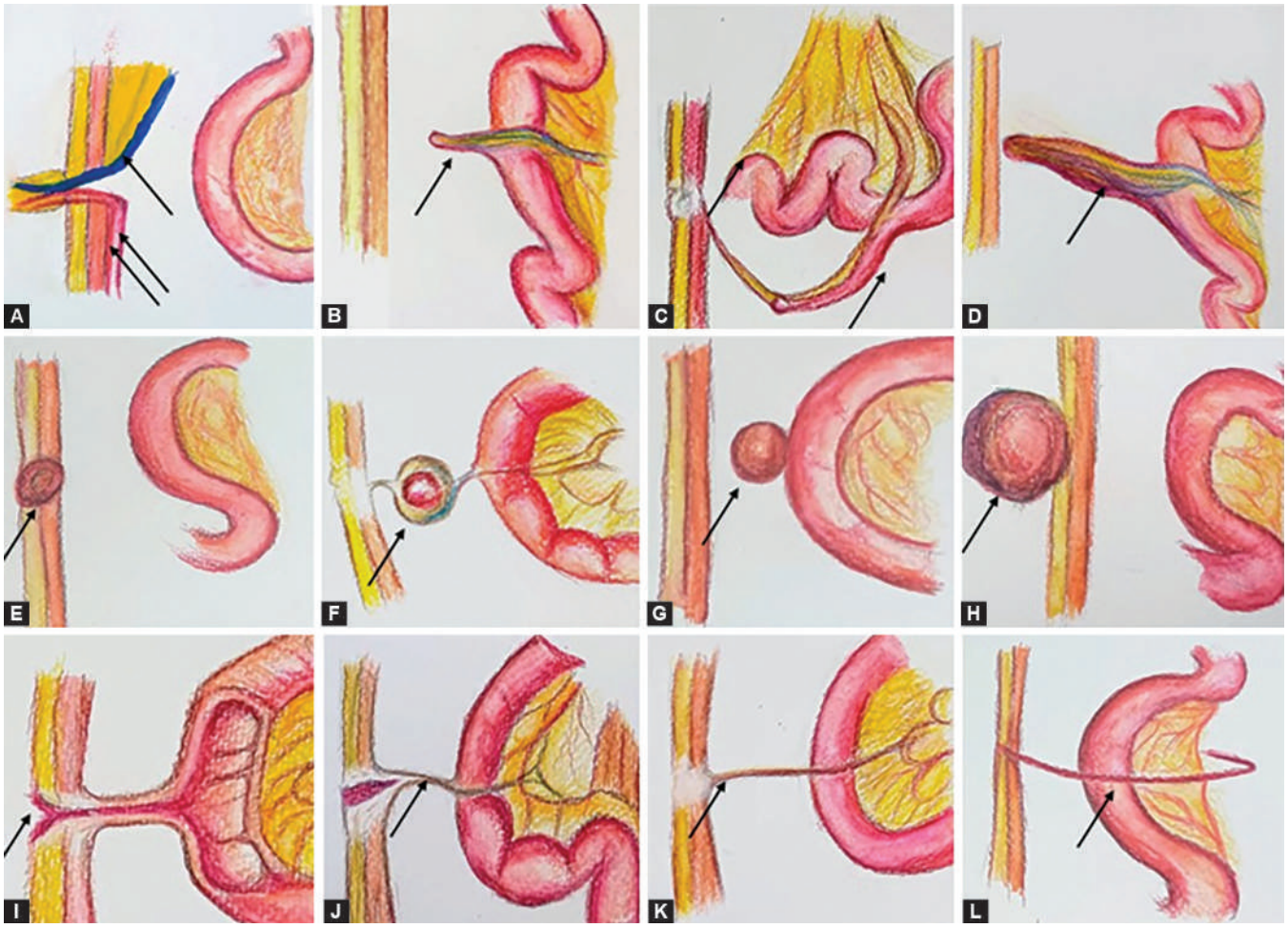
Meckel diverticulum has been associated with chromosomal abnormalities such as trisomy 13, trisomy 18, and trisomy 21. However, no specific genes have been identified as causative. These associations are thought to reflect the high prevalence of multiple congenital anomalies, including gastrointestinal malformations, cardiac defects, and craniofacial anomalies. Some patients show cecal duplication cysts in addition to MD.[24] It has also been associated with the broader patterns of multiple congenital anomalies, including the VACTERL association, omphalocele, gastroschisis, intestinal atresia, malrotation, and congenital cardiac defects such as ventricular and/or atrial septal defects, and less frequently, conotruncal anomalies.[3] Overall, MD is considered congenital but not genetically inherited, where the associations reflect disrupted embryologic development rather than a defined hereditary syndrome.

The heterotopia seen in MD does not have a clear relationship with specific Mendelian genes but may be related to developmental mis-patterning of the foregut-midgut boundary.[12,22] Gastric heterotopia has been associated with increased expression of the SRY-related HMG-box (SOX) 2, a key foregut transcription factor vital for maintaining stomach tissue identity and function.[25–27] Other gastric epithelial markers, such as mucin (MUC) 5AC in foveolar cells, MUC6 in pyloric glands, the alpha and beta subunits of the gastric H^+/K^+-ATPase (ATP4A, ATP4B), and pepsinogen in fundic-type heterotopia, are also notable in parietal and chief cell differentiation.[28–32] Current information links SOX2 expression with the presence of gastric characteristics, such as the presence of gastric mucins MUC5AC and MUC6, and its loss/ectopic expression with non-gastric mucosal patterning, metaplasia, or heterotopia.[33–35] The gel-like mucin glycoproteins are characteristically present in the stomach, duodenum, and pancreas, where these shield the epithelium from acid, proteases, and pathogens.[36,37] The gastric H^+/K^+-ATPases are the proton pumps in stomach parietal cells; these enzymes pump hydrogen ions (H^+) into the stomach lumen in exchange for potassium ions (K^+), creating the highly acidic environment needed for digestion.[32,38]

SRY-related HMG-box 2 expression shows a high-to-low anterior–posterior gradient within the foregut endoderm; the strongest expression is seen in the esophagus and stomach with a progressive downregulation toward the foregut–midgut junction.[39,40] This graded SOX2 expression helps establish foregut identity, promoting gastric and esophageal epithelial differentiation while actively repressing intestinal fate.[41,42] It suppresses intestinal gene programs by antagonizing *CDX2*; these changes are modulated by regional Wnt/β-catenin, BMP, FGF, and retinoic acid signaling gradients.[43,44] Disruption or persistence of SOX2 expression beyond its normal domain can shift epithelial patterning toward a gastric phenotype, contributing to developmental anomalies such as gastric heterotopia in the intestine.[43,44]

In contrast to SOX2, the gene *CDX2* likely promotes intestinal epithelial identity.[45,46] It induces hallmark intestinal genes such as *MUC2*, *villin (VIL1)*, sucrase-isomaltase (SI), and *KLF4* gene Krüppel-like factor 4 (*KLF4*, which encodes a transcription factor vital for cell proliferation, differentiation, and stem cell maintenance).[47,48] Consequently, *CDX2* represses gastric lineage factors such as SOX2 and drives goblet cell formation and intestinal-type architecture, reinforcing the phenotypic switch.[45,46,49]

Meckel diverticulums can show considerable inflammation. Genes implicated in inflammatory pathways, such as those in the toll-like receptor (TLR) and nod-like receptor (NLR) activation, have been examined. Toll-like receptors are not involved in the formation of MD, but these might be important in the exaggerated immune responses to bacterial or inflammatory stimuli.[1,3] Similarly, the NLR pathway mediators do not seem to be directly involved.[1,3] Nod-like receptor family CARD domain containing 4 (NLRC4), a known regulatory gene in the inflammasome-mediated immune



Figs 1A to L: (A) The normal umbilicus at birth is best recognized for its vascular connections. There are two umbilical arteries (red) ascending along the anterior abdominal wall (arrows). These carry blood from the internal iliac arteries in the pelvis and pass through the umbilicus to the placenta. After birth, the obliterated remnants of these arteries can be seen as the subperitoneal medial umbilical ligaments. The umbilical vein runs along the free edge of the falciform ligament, a bilayered, sickle-shaped fold of peritoneum that extends from the anterior abdominal wall to the inferior border and anterior surface of the liver. The vein undergoes fibrosis and can be seen later as the ligamentum teres hepatis (round ligament of the liver); (B–L) An MD and some other congenital defects in the umbilicus. The key findings in all the figures are marked by arrows: (B) Meckel diverticulum, a blind, isolated pouch is a remnant of the OMD that arises on the small intestine (ileum) and usually does not open into the umbilicus; (C) An MD with an associated fibrous cord that extends from the peritoneum to the umbilicus; (D) Meckel diverticulitis, with inflammation in the outpouching due to infection or enzymatic damage; (E) Umbilical polyp; (F) Fibrous umbilical cord with intra-abdominal cyst; (G) Duplication cyst from the small intestine. The neck is smaller than typically seen; (H) A large umbilical granuloma (these are usually <1 cm in size); (I) Umbilical-intestinal fistula (patent vitellointestinal duct); (J) Umbilical cord sinus; (K) Fibrous cord between the umbilicus and the small intestine; and (L) Remnant of umbilical vessel

responses, has been examined.[50] Its mutations have been implicated in autoinflammatory diseases like infantile enterocolitis; it could enhance inflammation in MD, but does not have a causative role. At the cellular level, macrophage activation can similarly enhance the local inflammatory responses.[4]

Pathophysiology

After birth, the OMD usually gets obliterated by 5 weeks of age. Meckel diverticulums have been seen associated with many related abnormalities: (1) an MD with the rest of the OMD seen as a terminal fibrous band connecting with the umbilicus. This fibrous connection can be complicated by intestinal obstruction, volvulus, or internal hernia; (2) the whole OMD remaining patent, seen as a fistulous connection from the ileum to the umbilicus with fecal discharge from the umbilicus; and (3) an umbilical cyst, a fluid-filled remnant in a part of the ductal pathway.[51–55] These, an inflamed MD, and a few other umbilical anomalies are shown in Figure 1.

Clinical Features

Meckel diverticulum is a true congenital outpouching of the ileum that arises from incomplete involution of the vitelline duct, not due to abnormal intestinal innervation.[6] Hence, many patients can remain completely asymptomatic. The diverticulum contains all layers of the intestinal wall, including the myenteric (Auerbach) and submucosal (Meissner) plexuses, so its intrinsic innervation is generally normal and should not be a primary cause of dysmotility.[3]

Considering that these congenital diverticula contain all layers of the intestinal wall, they should also not have an inherent tendency for scarring.[3]

In other patients, MD can be associated with other abnormalities (Fig. 1) and present with clinical complications. In some cases, remnants of the OMD can evolve over time into linear cord-like structures that connect the diverticulum to the umbilicus or abdominal wall.[53,55] These can alter gut motility by kinking, compressing, or entrapping loops of small bowel.[53] Many patients may show clinical features suggestive of inflammation, dysmotility, obstruction, volvulus, or intussusception, with the diverticulum acting as the lead point.[2,3,6,56,57] They may present with nausea, vomiting, low-grade fever, right lower quadrant abdominal pain, and painless rectal bleeding.[6–8] In some cases, chronic inflammation may lead to localized fibrosis and adhesions. Recognition of these bands is important, as surgical removal of the MD along with the band is typically required to prevent recurrent obstruction.[53]

The ectopic gastric tissue in many MDs secretes acid, and can cause peptic ulceration, bleeding, and inflammation (diverticulitis) within the diverticulum or in the nearby bowel.[5] These MDs frequently show visible/histopathological signs of inflammation (Figs 1B and D);[58,59] this condition, known as Meckel diverticulitis, can show clinical features similar to those of acute appendicitis.[60] Inflammation may also cause stasis of bowel contents within the diverticulum.

There is no direct link between MD and peripheral eosinophilia. However, secondary eosinophilia may occasionally occur if the diverticulum becomes inflamed or if there is ulceration from ectopic gastric tissue, leading to a local inflammatory response that can slightly elevate eosinophil counts.[61,62] Rarely, if the diverticulum contains ectopic pancreatic or other heterotopic tissue, it could provoke a localized immune reaction. Some infants show hypersensitivity-type reactions in the diverticular tissue.[3,62]

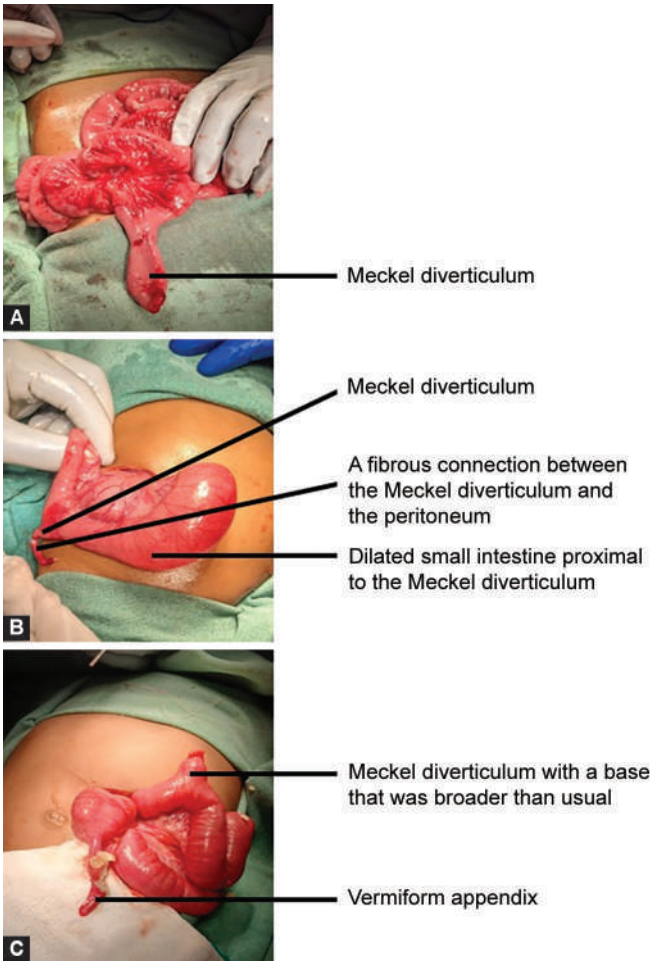
Fecal calprotectin is a biomarker of intestinal inflammation.[63] In MD, fecal calprotectin is not typically elevated unless there is complicated inflammation such as Meckel diverticulitis or ulceration from ectopic gastric tissue.[64,65]

Diagnosis

Meckel diverticulums are often found incidentally. Nuclear Meckel scans using intravenously administered technetium-99m pertechnetate, which is selectively taken up by true/ectopic gastric mucosal cells, can help in detecting gastric tissue.[66–68] This scan is useful in infants/children presenting with painless rectal bleeding or suspected ulceration due to acid secretion from ectopic gastric tissue.[67] Sensitivity rates of 85–90% have been documented in children. However, in adults, lower detection rates of about 60% have been seen because of smaller/inactive ectopic tissue.[68] There may be false positives in patients with intestinal inflammation or duplication cysts.[69,70] Others, those without gastric mucosa, show a false negative.[70] Overall, Meckel scans are considered the diagnostic test of choice for identifying symptomatic MDs with gastric mucosa.[68] In many cases, the diagnosis is often confirmed only after surgery with histopathological examination of the excised tissues.[5,71]

Treatment

Asymptomatic patients may be closely followed with conservative management.[72] However, for MDs discovered incidentally during a laparotomy or in symptomatic cases, including those with bleeding, obstruction, inflammation (diverticulitis), or perforation, a diverticulectomy is performed.[72–75] If the base is broad or there is associated ulceration or inflammation of the adjacent ileum, a segmental ileal resection is considered.[72,73] These procedures can be performed via open laparotomy or laparoscopically, depending on the clinical situation and the surgeons' experience.[75] In children presenting with bleeding, removal of the diverticulum usually resolves the problem.[75] However, while in cases with obstruction, surgical release of fibrous bands or adhesions may also be necessary.[55] Postoperative outcomes are generally excellent, with low recurrence and resolution of symptoms.[6,72]



Figs 2A to C: Intraoperative findings seen in three different infants with Meckel diverticula

Figure 2 shows the gross morphology of a newly resected MD from three different infants. Histopathological examination of these tissue samples typically shows gastric heterotopia with mucus-producing goblet cells and acid-producing parietal cells (Fig. 3).

There is no direct link between MD and neurodevelopmental disorders, and it does not inherently affect brain development or cognitive function.[6,76] However, in rare cases where MD occurs as part of chromosomal abnormalities or syndromic conditions, such as trisomy 13, 18, or 21, or VACTERL association, there may be associated neurodevelopmental delays or intellectual disabilities, but these outcomes are due to the underlying syndrome rather than the diverticulum itself.[76]

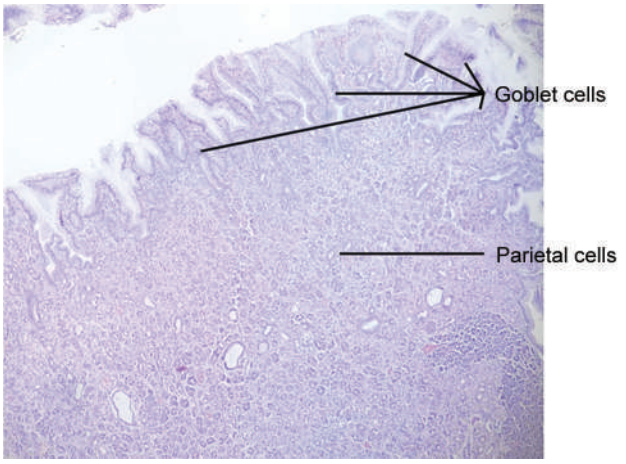


Fig. 3: Histopathological findings (hematoxylin-eosin, 20×) in a tissue section of an MD. Gastric heterotopia was seen with mucus-producing goblet cells and acid-producing parietal cells

Conclusion

Meckel diverticulum is one of the most common congenital gastrointestinal malformations all over the world. However, it still poses a diagnostic challenge in many infants due to its diverse clinical presentations, ranging from incidental findings to life-threatening complications. Its embryological origin explains the presence of ectopic tissue, which contributes to complications such as bleeding, inflammation, and intestinal obstruction. Advances in imaging and surgical techniques have improved outcomes, but timely diagnosis still remains important. A thorough understanding of its embryologic origin, clinical manifestations, and management strategies is essential for optimizing patient care.

Acknowledgment

The author would like to thank Dr Akhil Maheshwari, Professor of Pediatrics/Neonatology with the Boston Children's Health Physicians Group at New York Medical College/Maria Fareri Children's Hospital, Valhalla, New York, for all his help and encouragement in developing this manuscript.

References

1. Sagar J, Kumar V, Shah DK. Meckel's diverticulum: A systematic review. J R Soc Med 2006;99(10):501–505. DOI: 10.1177/014107680609901011.
2. Zhang R, Liu Y, An N. A single-center study of secondary intussusception in children with Meckel's diverticulum. BMC Pediatr 2025;25(1):922. DOI: 10.1186/s12887-025-06300-5.
3. An J, Zabbo CP. Meckel Diverticulum. Treasure Island, FL, USA: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK499960/.
4. Stallion A, Shuck JM. Meckel's diverticulum. In: Surgical Treatment: Evidence-Based and Problem-Oriented. Munich, Germany: Zuckschwerdt; 2001. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6918/.
5. Martin JP, Connor PD, Charles K. Meckel's diverticulum. Am Fam Physician 2000;61(4):1037–1042, 1044. PMID: 10706156.
6. Park JJ, Wolff BG, Tollefson MK, et al. Meckel diverticulum: The Mayo Clinic experience with 1476 patients (1950-2002). Ann Surg 2005;241(3):529–533. DOI: 10.1097/01.sla.0000154270.14308.5f.
7. Shaikh FA, Ka DV, Shaikh H, et al. Recognizing perforated Meckel's diverticulum: A crucial differential in acute appendicitis imitation. Clin Case Rep 2024;12(9):e9361. DOI: 10.1002/ccr3.9361.
8. Evola G, Caramma S, Caruso G, et al. Intestinal obstruction and ischemia by necrotic annular Meckel's diverticulum: Case report and review of the literature. Int J Surg Case Rep 2021;82:105897. DOI: 10.1016/j.ijscr.2021.105897.
9. Hansen CC, Soreide K. Systematic review of epidemiology, presentation, and management of Meckel's diverticulum in the 21st century. Medicine (Baltimore) 2018;97(35):e12154. DOI: 10.1097/MD.0000000000012154.
10. Celebi S. Male predominance in Meckel's diverticulum: A hyperacidity hypotheses. Med Hypotheses 2017;104:54–57. DOI: 10.1016/j.mehy.2017.05.014.
11. Chang YC, Lai JN, Chiu LT, et al. Epidemiology of Meckel's diverticulum: A nationwide population-based study in Taiwan: Characteristics of the cases from surgery between 1996 and 2013. Medicine (Baltimore) 2021;100(50):e28338. DOI: 10.1097/MD.0000000000028338.
12. Malone JC, Arbor TC, Shah AB. Embryology, Midgut. Treasure Island, FL, USA: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553156/.
13. Walters JR, Howard A, Rumble HE, et al. Differences in expression of homeobox transcription factors in proximal and distal human small intestine. Gastroenterology 1997;113(2):472–477. DOI: 10.1053/gast.1997.v113.pm9247466.
14. Suh E, Traber PG. An intestine-specific homeobox gene regulates proliferation and differentiation. Mol Cell Biol 1996;16(2):619–625. DOI: 10.1128/MCB.16.2.619.
15. Orzechowska-Licari EJ, Bialkowska AB, Yang VW. Sonic Hedgehog and WNT signaling regulate a positive feedback loop between intestinal epithelial and stromal cells to promote epithelial regeneration. Cell Mol Gastroenterol Hepatol 2023;16(4):607–642. DOI: 10.1016/j.jcmgh.2023.07.004.
16. Xie Z, Zhang M, Zhou G, et al. Emerging roles of the Hedgehog signalling pathway in inflammatory bowel disease. Cell Death Discov 2021;7(1):314. DOI: 10.1038/s41420-021-00679-7.
17. Danopoulos S, Schlieve CR, Grikscheit TC, et al. Fibroblast growth factors in the gastrointestinal tract: Twists and turns. Dev Dyn 2017;246(4):344–352. DOI: 10.1002/dvdy.24491.
18. Al Alam D, Danopoulos S, Schall K, et al. Fibroblast growth factor 10 alters the balance between goblet and Paneth cells in the adult mouse small intestine. Am J Physiol Gastrointest Liver Physiol 2015;308(8):G678–G690. DOI: 10.1152/ajpgi.00158.2014.
19. Auclair BA, Benoit YD, Rivard N, et al. Bone morphogenetic protein signaling is essential for terminal differentiation of the intestinal secretory cell lineage. Gastroenterology 2007;133(3):887–896. DOI: 10.1053/j.gastro.2007.06.066.
20. Shroyer NF, Wong MH. BMP signaling in the intestine: Cross-talk is key. Gastroenterology 2007;133(3):1035–1038. DOI: 10.1053/j.gastro.2007.07.018.
21. Liu J, Xiao Q, Xiao J, et al. Wnt/beta-catenin signalling: Function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther 2022;7(1):3. DOI: 10.1038/s41392-021-00762-6.
22. Malik AA, Shams ul B, Wani KA, et al. Meckel's diverticulum-Revisited Saudi J Gastroenterol 2010;16(1):3–7. DOI: 10.4103/1319-3767.58760.
23. Yahchouchy EK, Marano AF, Etienne JC, et al. Meckel's diverticulum. J Am Coll Surg 2001;192(5):658–662. DOI: 10.1016/s1072-7515(01)00817-1.
24. Hakimi T, Aslamzai M, Seyar F, et al. Coexistence of cecal duplication cyst and Meckel's diverticulum presenting as intussusception in a malnourished child a case report with literature review BMC Pediatr 2025;25(1):479. DOI: 10.1186/s12887-025-05823-1.
25. Li XL, Eishi Y, Bai YQ, et al. Expression of the SRY-related HMG box protein SOX2 in human gastric carcinoma. Int J Oncol 2004;24(2):257–263. PMID: 14719100.
26. Huang Q, Liu L, Zhan L, et al. Heterotopic gastric mucosa in the second part of the duodenum causing hemorrhage in a child: A case report and literature review. Front Pediatr 2025;13:1641000. DOI: 10.3389/fped.2025.1641000.
27. Niharika, Ureka L, Roy A, et al. Dissecting SOX2 expression and function reveals an association with multiple signaling pathways during embryonic development and in cancer progression. Biochim

Biophys Acta Rev Cancer 2024;1879(5):189136. DOI: 10.1016/j.bbcan.2024.189136.
28. Babu SD, Jayanthi V, Devaraj N, et al. Expression profile of mucins (MUC2, MUC5AC and MUC6) in Helicobacter pylori infected preneoplastic and neoplastic human gastric epithelium. Mol Cancer 2006;5:10. DOI: 10.1186/1476-4598-5-10.
29. Reis CA, David L, Correa P, et al. Intestinal metaplasia of human stomach displays distinct patterns of mucin (MUC1, MUC2, MUC5AC, and MUC6) expression. Cancer Res 1999;59(5):1003–1007. PMID: 10070955.
30. Singh V, Mani I, Chaudhary DK. ATP4A gene regulatory network for fine-tuning of proton pump and ion channels. Syst Synth Biol 2013;7(1–2):23–32. DOI: 10.1007/s11693-012-9103-1.
31. Khan S, Niaz S, Khan M, et al. A case of heterotopic gastric tissue in duodenal bulb: An interesting endoscopic finding. Cureus 2022;14(4):e24271. DOI: 10.7759/cureus.24271.
32. Engevik AC, Kaji I, Goldenring JR. The physiology of the gastric parietal cell. Physiol Rev 2020;100(2):573–602. DOI: 10.1152/physrev.00016.2019.
33. Tsukamoto T, Mizoshita T, Mihara M, et al. Sox2 expression in human stomach adenocarcinomas with gastric and gastric-and-intestinal-mixed phenotypes. Histopathology 2005;46(6):649–658. DOI: 10.1111/j.1365-2559.2005.02170.x.
34. Park ET, Gum JR, Kakar S, et al. Aberrant expression of SOX2 upregulates MUC5AC gastric foveolar mucin in mucinous cancers of the colorectum and related lesions. Int J Cancer 2008;122(6):1253–1260. DOI: 10.1002/ijc.23225.
35. Marques T, David L, Reis C, et al. Topographic expression of MUC5AC and MUC6 in the gastric mucosa infected by Helicobacter pylori and in associated diseases. Pathol Res Pract 2005;201(10):665–672. DOI: 10.1016/j.prp.2005.03.007.
36. Dharmani P, Srivastava V, Kissoon-Singh V, et al. Role of intestinal mucins in innate host defense mechanisms against pathogens. J Innate Immun 2009;1(2):123–135. DOI: 10.1159/000163037.
37. Johansson ME, Sjovall H, Hansson GC. The gastrointestinal mucus system in health and disease. Nat Rev Gastroenterol Hepatol 2013;10(6):352–361. DOI: 10.1038/nrgastro.2013.35.
38. Sachs G, Shin JM, Vagin O, et al. The gastric H,K ATPase as a drug target: Past, present, and future. J Clin Gastroenterol 2007;41(Suppl 2):S226–S242. DOI: 10.1097/MCG.0b013e31803233b7.
39. Que J, Okubo T, Goldenring JR, et al. Multiple dose-dependent roles for Sox2 in the patterning and differentiation of anterior foregut endoderm. Development 2007;134(13):2521–2531. DOI: 10.1242/dev.003855.
40. Hagey DW, Klum S, Kurtsdotter I, et al. SOX2 regulates common and specific stem cell features in the CNS and endoderm derived organs. PLoS Genet 2018;14(2):e1007224. DOI: 10.1371/journal.pgen.1007224.
41. Jin RU, Xu Y, Lih TM, et al. SOX2 regulates foregut squamous epithelial homeostasis and is lost during Barrett's esophagus development. J Clin Invest 2025;135(16):e190374. DOI: 10.1172/JCI190374.
42. Zhang J, Wang Z, Zhao H, et al. The roles of the SOX2 protein in the development of esophagus and esophageal squamous cell carcinoma, and pharmacological target for therapy. Biomed Pharmacother 2023;163:114764. DOI: 10.1016/j.biopha.2023.114764.
43. Raghoebir L, Bakker ERM, Mills JC, et al. SOX2 redirects the developmental fate of the intestinal epithelium toward a premature gastric phenotype. J Mol Cell Biol 2012;4(6):377–385. DOI: 10.1093/jmcb/mjs030.
44. Mutoh H, Sashikawa M, Sugano K. Sox2 expression is maintained while gastric phenotype is completely lost in Cdx2-induced intestinal metaplastic mucosa. Differentiation 2011;81(2):92–98. DOI: 10.1016/j.diff.2010.10.002.
45. Gao N, White P, Kaestner KH. Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. Dev Cell 2009;16(4):588–599. DOI: 10.1016/j.devcel.2009.02.010.
46. Crissey MAS, Guo RJ, Funakoshi S, et al. Cdx2 levels modulate intestinal epithelium maturity and Paneth cell development. Gastroenterology 2011;140(2):517–528.e8. DOI: 10.1053/j.gastro.2010.11.033.
47. Dang DT, Mahatan CS, Dang LH, et al. Expression of the gut-enriched Kruppel-like factor (Kruppel-like factor 4) gene in the human colon cancer cell line RKO is dependent on CDX2. Oncogene 2001;20(35):4884–4890. DOI: 10.1038/sj.onc.1204645.
48. Ghaleb AM, Yang VW. Kruppel-like factor 4 (KLF4): What we currently know. Gene 2017;611:27–37. DOI: 10.1016/j.gene.2017.02.025.
49. Guo RJ, Suh ER, Lynch JP. The role of Cdx proteins in intestinal development and cancer. Cancer Biol Ther 2004;3(7):593–601. DOI: 10.4161/cbt.3.7.913.
50. Matico RE, Yu X, Miller R, et al. Structural basis of the human NAIP/NLRC4 inflammasome assembly and pathogen sensing. Nat Struct Mol Biol 2024;31(1):82–91. DOI: 10.1038/s41594-023-01143-z.
51. Clemente EJI, Navarro OM, Irujo MN, et al. Omphalomesenteric duct anomalies in children: A multimodality overview. Radiographics 2021;41(7):2090–2110. DOI: 10.1148/rg.2021210048.
52. Morita K, Haga Y, Miyanari N, et al. A case of an omphalomesenteric duct remnant in an adult treated with laparoscopic surgery. Int J Surg Case Rep 2015;8C:179–181. DOI: 10.1016/j.ijscr.2015.01.047.
53. Sun C, Hu X, Huang L. Intestinal obstruction due to congenital bands from vitelline remnants: Sonographic features and review of the literature. J Ultrasound Med 2012;31(12):2035–2038. DOI: 10.7863/jum.2012.31.12.2035.
54. Markogiannakis H, Theodorou D, Toutouzas KG, et al. Persistent omphalomesenteric duct causing small bowel obstruction in an adult. World J Gastroenterol 2007;13(15):2258–2260. DOI: 10.3748/wjg.v13.i15.2258.
55. Kleiner O, Cohen Z, Finaly R, et al. Unusual presentation of omphalomesenteric duct remnant: A variant of mesodiverticular band causing intestinal obstruction. J Pediatr Surg 2000;35(7):1136–1137. DOI: 10.1053/jpsu.2000.7847.
56. Bouassida M, Feidi B, Ben Ali M, et al. Intussusception caused by an inverted Meckel's diverticulum: A rare cause of small bowel obstruction in adults. Pan Afr Med J 2011;10:57. PMID: 22384303.
57. Craine S, Agarwal S, Al-Hamid Z. Intussusception of Meckel's Diverticulum: A Case Report. Cureus 2024;16(12):e76513. DOI: 10.7759/cureus.76513.
58. Chen JJ, Lee HC, Yeung CY, et al. Meckel's Diverticulum: Factors Associated with Clinical Manifestations. ISRN Gastroenterol 2014;2014:390869. DOI: 10.1155/2014/390869.
59. Keese D, Rolle U, Gfroerer S, et al. Symptomatic Meckel's diverticulum in pediatric patients-case reports and systematic review of the literature. Front Pediatr 2019;7:267. DOI: 10.3389/fped.2019.00267.
60. Emre A, Akbulut S, Yilmaz M, et al. Double Meckel's diverticulum presenting as acute appendicitis: A case report and literature review. J Emerg Med 2013;44(4):e321–e324. DOI: 10.1016/j.jemermed.2012.11.001.
61. Corrigan B, Bursle GA. Eosinophilic Meckel's diverticulitis. Intern Med J 2002;32(11):564–565. DOI: 10.1046/j.1445-5994.2002.00283.x.
62. Choi SY, Hong SS, Park HJ, et al. The many faces of Meckel's diverticulum and its complications. J Med Imaging Radiat Oncol 2017;61(2):225–231. DOI: 10.1111/1754-9485.12505.
63. Konikoff MR, Denson LA. Role of fecal calprotectin as a biomarker of intestinal inflammation in inflammatory bowel disease. Inflamm Bowel Dis 2006;12(6):524–534. DOI: 10.1097/00054725-200606000-00013.
64. Kanungo A, Shenoy S, D'Cunha A, et al. Meckel's diverticulum mimicking inflammatory bowel disease. J Health Allied Sci 2023;13(1):294–296. DOI: 10.1055/s-0042-1757966.
65. Kang HS, Lee JS, Hyun CR, et al. Meckel's diverticulum diagnosed in a child with suspected small bowel Crohn's disease. Pediatr Gastroenterol Hepatol Nutr 2019;22(1):98–104. DOI: 10.5223/pghn.2019.22.1.98.
66. Jaros R, Schussheim A, Levy LM. Preoperative diagnosis of bleeding Meckel's diverticulum utilizing 99m technetium pertechnetate scinti-imaging. J Pediatr 1973;82(1):45–49. DOI: 10.1016/s0022-3476(73)80008-3.
67. Leonidas JC, Germann DR. Technetium-99m pertechnetate imaging in diagnosis of Meckel's diverticulum. Arch Dis Child 1974;49(1):21–26. DOI: 10.1136/adc.49.1.21.

68. Titley-Diaz WH, Aziz M. Meckel Scan. Treasure Island, FL, USA: StatPearls Publishing; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560500/.
69. Wise PA, Sagar VV. False-positive Meckel's scan in a case of infected ovarian cyst. Eur J Nucl Med 1985;10(9-10):462–463. DOI: 10.1007/BF00256592.
70. Schwartz MJ, Lewis JH. Meckel's diverticulum: Pitfalls in scintigraphic detection in the adult. Am J Gastroenterol 1984;79(8):611–618. PMID: 6087654.
71. Ahuja S, Malik S, Zaheer S. Meckel's diverticulum with ectopic pancreatic and epididymal tissue in a newborn infant. Int J Surg Case Rep 2025;128:111048. DOI: 10.1016/j.ijscr.2025.111048.
72. Snyder CL. Current management of umbilical abnormalities and related anomalies. Semin Pediatr Surg 2007;16(1):41–49. DOI: 10.1053/j.sempedsurg.2006.10.006.
73. Kumar HR. An update on the management of Meckel's diverticulum: Review Article SAR J Surg 2025;6(3):57–61. DOI: 10.36346/sarjs.2025.v06i03.005.
74. Varcoe RL, Wong SW, Taylor CF, et al. Diverticulectomy is inadequate treatment for short Meckel's diverticulum with heterotopic mucosa. ANZ J Surg 2004;74(10):869–872. DOI: 10.1111/j.1445-1433.2004.03191.x.
75. Robinson JR, Correa H, Brinkman AS, et al. Optimizing surgical resection of the bleeding Meckel diverticulum in children. J Pediatr Surg 2017;52(10):1610–1615. DOI: 10.1016/j.jpedsurg.2017.03.047.
76. Chen Q, Gao Z, Zhang L, et al. Multifaceted behavior of Meckel's diverticulum in children. J Pediatr Surg 2018;53(4):676–681. DOI: 10.1016/j.jpedsurg.2017.11.059.

REVIEW ARTICLE

Principal Component Analysis: Progress and Applications

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Received on: 10 December 2025; *Accepted on*: 02 February 2026; *Published on*: 08 April 2026

Abstract

Principal component analysis (PCA) is a statistical technique used to reduce the dimensionality of large datasets while preserving as much important information as possible. It performs a mathematical rotation of the data to create a new set of uncorrelated variables called principal components (PCs), which are ordered by the amount of variance explained in the data and are orthogonal (uncorrelated) to each other. Principal component analysis is used for analysis because such data typically involves thousands of gene expression variables measured across a relatively small number of samples. As each gene represents one dimension, a dataset could potentially represent a 10,000–30,000-dimensional space. Principal component analysis then reduces this high dimensionality by transforming the original gene expression variables into a smaller set of PCs that capture the majority of variation in the data. This helps researchers visualize complex expression patterns, identify clusters of samples, detect outliers, and uncover underlying biological differences between healthy and diseased states. Principal component analysis also helps reduce noise and redundancy in microarray datasets, making downstream statistical analysis and classification more reliable and efficient. In this review, the authors have reviewed current information on the generation of PCs and then subsequent use/analysis of data generated from microarray or similar large data sources.

Keywords: Background correction, Correlation matrix, Covariance matrix, Data centering, Data noise, Dimensionality, Eigenvalues, Eigenvectors, Exponential normalization, Gene expression, GEO matrices, High-throughput datasets, Infant, Log_2 transformation, Machine learning, Microarray, Neonate, Newborn, Normalization, Outliers, Preprocessing, Preprocessing of data, Principal component scores, Quantile normalization, Redundancy, Robust multi-array averages, Variation.

Newborn (2026): 10.5005/jp-journals-11002-0150

Keypoints

- Principal component analysis (PCA) is a statistical technique used to reduce the dimensionality of large datasets while preserving as much important information as possible.
- In microarray data, PCA is used for analysis because such data typically involves thousands of gene expression variables measured across a relatively small number of samples.
- Principal component analysis compresses data from thousands of genes to a handful of components, where the computed principal components (PCs) represent a progressively decreasing degree of variation.
- This article provides a review of current methods of generation of principal components, including preprocessing of data with normalization, log_2 transformation, and, if needed, scaling. Information is also included about centering of data, computation of covariance or correlation matrices, calculation of eigenvalues and eigenvectors, selection of PC, and analysis of results.

Introduction

Principal component analysis is a statistical technique used to reduce the dimensionality of large datasets while preserving as much important information as possible.[1–8] It performs a mathematical rotation of the data to create a new set of uncorrelated variables called PCs, which are ordered by the amount of variance explained in the data and are orthogonal (uncorrelated) to each other.[8,9] The PCs are ordered by the degree of variance that is explained by each.[3] By keeping only the top PCs, PCA simplifies complex datasets, reduces noise, improves visualization, and can

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make machine learning models faster and more effective without significantly losing information.[10,11]

In microarray/other high-throughput physiological data, PCA is used for analysis because such information typically carries thousands of gene expression variables measured across a relatively small number of samples.[5,6,12–14] As each gene represents one dimension, and therefore, a dataset could possibly represent a 10,000–30,000-dimensional space.[15] Principal component analysis reduces this high dimensionality by transforming the original gene expression variables into a smaller set of PCs that captures the majority of variation in the data.[12] This helps researchers visualize complex expression patterns, identify clusters of samples, detect outliers, and uncover underlying biological differences between health and diseased states.[9,13,16] Additionally, PCA helps reduce noise and redundancy in microarray datasets, making downstream statistical analysis and classification more reliable and efficient.[17]

After the collection of a large number of data points, PCA then compresses all the information to a handful of components, where the computed PCs represent a progressively decreasing degree of variation.[7,12,13] PC1 + PC2 can explain 30–60% of total variance, and the first 5–10 PCs capture most of the biologically-meaningful variation.[8] Thus, the PCA compresses the data from thousands of genes to a small, manageable number of variables. In this review, we have attempted to explain the process by which PCs are generated and then used for the analysis of data generated from microarray or similar large data sources. We have combined some data from our own preliminary studies with an extensive literature search in Embase, PubMed, and Scopus.[18,19] To avoid bias in the identification of studies, keywords were short-listed *a priori* from PubMed's Medical Subject Headings (MeSH) thesaurus.[20]

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How to cite this article: Maheshwari A, Das JK, Guaragni B, *et al*. Principal Component Analysis: Progress and Applications. Newborn 2026;5(1):20–25.

Source of support: Nil

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

Generation of Principal Components

Principal component analysis is a standard exploratory step used to understand global patterns in high-throughput data such as those from microarray experiments, which might include changes in the expression of thousands of genes.[8,12–14,17,21] By reducing the dimensionality of the data, PCA can help identify dominant patterns of gene expression, detect outliers, and reveal underlying biological variation such as differences between experimental conditions or sample groups.[5,22,23] Principal component analysis reduces these high-dimensional data into a few principal components that capture the largest sources of variation, allowing researchers to visualize sample similarities and differences.[5,22,23] Overall, these analytical steps can help differentiate true biological signals from experimental noise, making it an essential quality-control and exploratory step before downstream steps.[1,8,11] Principal component analysis can also help in visualizing complex high-throughput datasets in 2–3 dimensions, making it easier for interpretation of results, assessment of data quality, and reduction of noise before applying further statistical or machine learning analyses.[10,24]

Principal Component Analysis (PCA)

The main steps in PCA are as follows:[1,3–5,14,21,23]

(a) Generation of principal components:

- Preprocessing of the data: Normalization, $\log_2$ transformation, and, if needed, scaling.
- Center the data: Subtraction of the mean of each variable.
- Compute covariance or correlation matrix: Measure relationships between variables.
- Calculate eigenvalues and eigenvectors: Identify directions of maximum variance.

(b) Selection of principal components: Choose components with the largest eigenvalues.

(c) Projection of data: Transform the original data into the principal component space.

(d) Interpretation of results: Analyze score and loading plots to understand patterns.

Generation of Principal Components

To transform the original variables in a dataset into PCs, the first step is to standardize the data so that each variable has a mean of zero and a standard deviation of one. This ensures that variables measured on different scales contribute equally.[3,25] Next, the covariance (or correlation) matrix of standardized data is computed to understand how variables vary together.

(a) Preprocessing of raw microarray data helps remove technical noise and make gene expression measurements comparable across samples.[26,27] It typically includes background correction to eliminate non-specific signal, normalization to adjust for systematic differences between arrays, and $\log_2$ transformation to stabilize variance across expression levels.[28] Additional steps may involve probe summarization and filtering out low-quality or low-expressed genes.[29] Proper preprocessing ensures that downstream analyses such as PCA and differential gene expression reflect true biological variation, not artifacts introduced during sample preparation, hybridization, or scanning.[12,30]

Table 1: Impact of background correction on PCs

Without background correction	*With background correction*
PCs dominated by noise	PCs reflect biology
Poor replicate clustering	Improved clustering
Artificial outliers	Reduced false outliers

PCs, principal components

Table 2: Effect of normalization on PCA results

Without normalization	*With normalization*
PCs dominated by intensity	PCs reflect condition differences
Replicates poorly cluster	Replicates cluster tightly
Artificial batch effects	Reduced batch effects

PCs, principal components; PCA, principal component analysis

(b) Background correction can help because raw data often contain technical noise, biases, and measurement errors that can distort the true structure of the dataset.[11] Background correction, along with normalization and scaling, helps ensure that the observed variation reflects meaningful biological or experimental differences rather than artifacts.[31] Applying PCA after these preprocessing steps allows the principal components to capture genuine patterns in the data, leading to more accurate dimensionality reduction, clearer visualization, and more reliable interpretation of results (Table 1).[3,5]

(c) Normalization is essential before performing PCA on high-throughput data.[3,32] Principal component analysis is variance-driven, and consequently, technical inconsistency between arrays could overtake biological variation if normalization is skipped or done improperly.[3,33] It is also needed because of potential problems arising from irregularities in labeling, scanner sensitivity, and hybridization (Table 2).[3,22,33,34]

Exponential normalization using negative controls (NEQC) is a frequently-used preprocessing method for background correction and normalization.[11,32] It uses the signal from negative control probes to estimate and remove background noise more accurately than simple subtraction methods. After background correction, NEQC typically applies $\log_2$ transformation and quantile normalization to make expression values comparable across samples.[35,36] This approach improves data quality by reducing technical variation, stabilizing variance, and ensuring that downstream analyses such as PCA reflect true biological differences rather than experimental artifacts.

Normalization with robust multi-array averages (RMAs) can further help with technical noise, background signals, and systematic biases that can distort the true biological variation.[9,37,38] robust multi-array average preprocessing corrects for background, normalizes across arrays, and summarizes probe-level data into expression measures, making the data consistent and comparable.[39,40] Conducting PCA on such preprocessed and normalized data helps detect genuine biological patterns, improves visualization, and supports more accurate clustering and downstream analyses.[41,42]

Quantile normalization is another normalization method; it adjusts the data so that all samples have the same overall distribution of expression values, making these comparable.[43,44] Performing PCA on normalized data ensures that the PCs capture true biological variation rather than technical artifacts, leading to more accurate dimensionality reduction, better visualization, and reliable interpretation of patterns and relationships in the dataset.[45] Quantile normalization may need to be avoided if there are global, biologically meaningful shifts in expression or highly unbalanced experimental designs.[46] However, this situation is not seen frequently.

(d) Log_2 transformation is often combined with PCA in the analysis of gene expression data.[47] It stabilizes the variance across a wide range of expression values and reduces the impact of extreme values or skewed distributions.[48] The data become more symmetric in a normal-like distribution, which is important because PCA relies on variance to identify principal components.[48,49] Log_2 transformation makes PCA analyses more accurate in identifying the underlying biological patterns, shows better clustering, and prevents highly-expressed outlier genes from dominating the principal components.[47,48]

Background correction can possibly be skipped in very high-quality arrays, preprocessed data such as in public GEO matrices, or when correction increases variance in low-expression probes.[50] However, skipping background correction is generally discouraged because PCA emphasizes variance, and noise inflates variance disproportionately.[3]

(e) Centering of the data: For centering data, the mean of each variable is subtracted from all its values so that each variable has a mean of zero.[8] Ensuring that a PCA will capture the variance around the average, not the absolute values, allows the analysis to focus on patterns of variation and relationships between variables instead of their raw magnitudes.[8]

(f) Covariance or correlation matrices: These are computed to quantify how variables vary together across observations.[3,45] The covariance matrix is used when variables are on the same scale, while the correlation matrix is preferred when variables have different scales.[3,45] The covariance matrix summarizes how pairs of variables vary together after the data have been mean-centered.[51] Each diagonal element represents the variance of a variable, while off-diagonal elements represent the covariance between variables.[52] The covariance matrix is used to compute eigenvalues and eigenvectors, which determine the principal components and the amount of variance each component explains.[3,53]

A correlation matrix measures the standardized relationships between variables after mean-centering and scaling.[3,53] It is particularly useful for variables measured on different scales, ensuring each variable contributes equally to the analysis. The correlation matrix is then used to compute eigenvalues and eigenvectors, which help define the PCs and measure variance.[3,54]

(g) Eigenvalues and eigenvectors: Principal component analysis uses linear algebra to transform data into PCs. It finds these by calculating eigenvectors (directions) and eigenvalues (importance) from the covariance matrix.[3,53] Principal component analysis selects the top components with the highest eigenvalues and projects the data onto them simplify the dataset.

The prefix "eigen" indicates a value or direction that is characteristic of a particular transformation or matrix.[53] An eigenvalue is a scalar value in a square matrix; it indicates how much the direction of its corresponding eigenvector is stretched or scaled when a linear transformation is applied.[3,53] In mathematical terms, for a matrix A, an eigenvalue λ, and a non-zero eigenvector v satisfy the equation $Av = \lambda v$.[3,53]

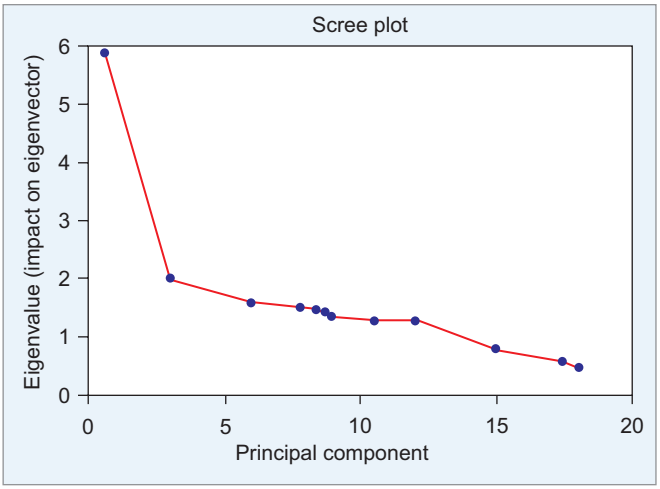

Fig. 1: Principal component analysis is an unsupervised machine learning technique that seeks to find PCs, predictor variables that explain the variation in a dataset. A scree plot shows the potency of each PC in terms of the change in the angle of the eigenvector
The prefix "eigen" indicates a scalar unit value that measures a particular vectorial transformation.

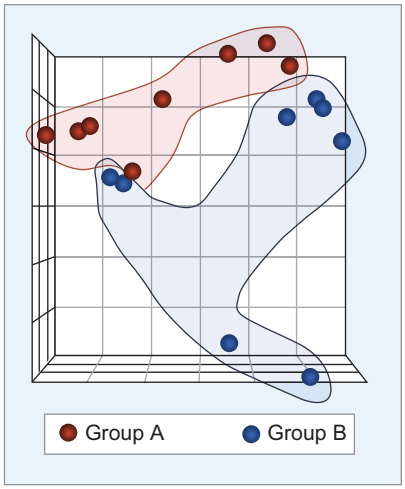

Fig. 2: Microarray profiles of two groups, A and B ($n = 8$ animals in each group). The graph is a scatter plot of the values of three PCs based on the correlation matrix of the total normalized array intensity data; each point represents one animal

Eigenvalues are important because they reveal key properties of a transformation or dataset, stability in systems, and patterns in many scientific applications.[3,45]

An eigenvector defines a direction of change when a linear transformation is applied to it.[3,45,55] For a square matrix A, the equation Av = the product of the eigenvalue λ and the direction of change defined by eigenvector v, λv, should be valid.[55,56] In data science, eigenvectors represent the directions of maximum variance.[3] The principal components are the top selected eigenvectors.[3,17,45,53]

Selection of Principal Components

Principal component analysis should ideally be applied to background-corrected, normalized, and log-transformed data, as technical noise can otherwise unduly dominate the principal components.[11,28] At this stage, the data are clean, comparable, and centered, allowing PCA to accurately capture meaningful patterns and sources of variation in the dataset.

Principal components are selected for the largest eigenvalues, as these selected variables should explain the greatest amount of variance in the data (Fig. 1).[3,45] The original data are transformed into the principal component space by multiplying them with the selected eigenvectors, resulting in principal component scores that represent the data in a reduced-dimensional form.[3,45]

Principal component scores are the coordinates of the original observations (samples) in the new PC space.[3,57] These are obtained by projecting the impact of selected eigenvectors on centered (and possibly scaled) data.[3,45,57] These scores summarize each observation in terms of the PCs, allowing visualization, clustering, and analysis of patterns in a lower-dimensional space while retaining most of the original variance (Fig. 2).[3,9,22,45,57]

Principal component analysis can help filter low-expression or low-variance genes by highlighting the genes that contribute most to the variation in the dataset.[12,58] In a gene expression analysis, genes with very low expression or little variation across samples often add noise rather than meaningful information.[12] In PCA, these genes typically contribute very little to the principal components, making it easier to identify and focus on high-variance genes that drive biological differences. This filtering improves downstream analyses, enhances visualization, and increases the reliability of clustering or classification results.[59]

Conclusion

Continued progress of PCA over the last several years underscores its importance in data analysis and machine learning. As datasets grow larger and more complex, PCA remains a reliable method for reducing dimensionality while preserving meaningful patterns. It is a critical tool for extracting valuable insights from high-dimensional data.

References

1. Wang W, Krishnan E. Big data and clinicians: A review on the state of the science. JMIR Med Inform 2014;2(1):e1. DOI: 10.2196/medinform.2913.
2. Jang JH. Principal component analysis of hybrid functional and vector data. Stat Med 2021;40(24):5152–5173. DOI: 10.1002/sim.9117.
3. Jolliffe IT, Cadima J. Principal component analysis: A review and recent developments. Philos Trans A Math Phys Eng Sci 2016;374(2065):20150202. DOI: 10.1098/rsta.2015.0202.
4. Konishi T. Principal component analysis for designed experiments. BMC Bioinformatics 2015;16(Suppl 18):S7. DOI: 10.1186/1471-2105-16-S18-S7.
5. Lever J, Krzywinski M, Altman N. Principal component analysis. Nat Methods 2017;14(7):641–642. DOI: 10.1038/nmeth.4346.
6. Liu C, Zhang X, Nguyen TT, et al. Partial least squares regression and principal component analysis: Similarity and differences between two popular variable reduction approaches. Gen Psychiatr 2022;35(1):e100662. DOI: 10.1136/gpsych-2021-100662.
7. McWhinney SR, Hlinka J, Bakstein E, et al. Principal component analysis as an efficient method for capturing multivariate brain signatures of complex disorders-ENIGMA study in people with bipolar disorders and obesity. Hum Brain Mapp 2024;45(8):e26682. DOI: 10.1002/hbm.26682.

8. Zhang Z, Castello A. Principal components analysis in clinical studies. Ann Transl Med 2017;5(17):351. DOI: 10.21037/atm.2017.07.12.
9. Nasser AA, Aljober MN, Alghawli ASA, et al. Revealing principal components, patterns, and structural gaps in health security among high-income countries: A comparative analysis using PCA and a multi-scenario clustering approach. F1000Res 2025;14:769. DOI: 10.12688/f1000research.168082.2.
10. Čikovský S, Šváb P, Hanák P. Hybrid PCA-based and machine learning approaches for signal-based interference detection and anomaly classification under synthetic data conditions. Sensors (Basel) 2025;25(24). DOI: 10.3390/s25247585.
11. Stanley JS, 3rd, Yang J, Li R, et al. Principled PCA separates signal from noise in omics count data. bioRxiv. DOI: 10.1101/2025.02.03.636129.
12. Ma S, Dai Y. Principal component analysis based methods in bioinformatics studies. Brief Bioinform 2011;12(6):714–722. DOI: 10.1093/bib/bbq090.
13. Price AL, Patterson NJ, Plenge RM, et al. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38(8):904–909. DOI: 10.1038/ng1847.
14. Sainani KL. Introduction to principal components analysis. PMR 2014;6(3):275–278. DOI: 10.1016/j.pmrj.2014.02.001.
15. Clarke R, Ressom HW, Wang A, et al. The properties of high-dimensional data spaces: Implications for exploring gene and protein expression data. Nat Rev Cancer 2008;8(1):37–49. DOI: 10.1038/nrc2294.
16. Qiu P, Gentles AJ, Plevritis SK. Discovering biological progression underlying microarray samples. PLoS Comput Biol 2011;7(4):e1001123. DOI: 10.1371/journal.pcbi.1001123.
17. Raychaudhuri S, Stuart JM, Altman RB. Principal components analysis to summarize microarray experiments: Application to sporulation time series. Pac Symp Biocomput 2000:455–466. DOI: 10.1142/9789814447331_0043.
18. Frandsen TF, Eriksen MB, Hammer DMG, et al. Using Embase as a supplement to PubMed in Cochrane reviews differed across fields. J Clin Epidemiol 2021;133:24–31. DOI: 10.1016/j.jclinepi.2020.12.022.
19. Burnham JF. Scopus database: A review. Biomed Digit Libr 2006;3:1. DOI: 10.1186/1742-5581-3-1.
20. Fremer E. Understanding MeSH for literature searches. JAMA 1995;273(3):184; author reply 184–185. DOI: 10.1001/jama.1995.03520270018013.
21. Ringnér M. What is principal component analysis? Nat Biotechnol 2008;26(3):303–304. DOI: 10.1038/nbt0308-303.
22. Groth D, Hartmann S, Klie S, et al. Principal components analysis. Methods Mol Biol 2013;930:527–547. DOI: 10.1007/978-1-62703-059-5_22.
23. Jonnalagadda S, Srinivasan R. Principal components analysis based methodology to identify differentially expressed genes in time-course microarray data. BMC Bioinformatics 2008;9:267. DOI: 10.1186/1471-2105-9-267.
24. Traidl S, Mathes S, Seurig S. AI - one size fits all? Allergol Select 2025;9:75–79. DOI: 10.5414/ALX02568E.
25. Petersen AH, Markussen B, Christensen KB. Exploratory data structure comparisons: Three new visual tools based on principal component analysis. J Appl Stat 2021;48(9):1675–1695. DOI: 10.1080/02664763.2020.1773772.
26. Owzar K, Barry WT, Jung SH, et al. Statistical challenges in preprocessing in microarray experiments in cancer. Clin Cancer Res 2008;14(19):5959–5966. DOI: 10.1158/1078-0432.CCR-07-4532.
27. Durinck S. Pre-processing of microarray data and analysis of differential expression. Methods Mol Biol 2008;452:89–110. DOI: 10.1007/978-1-60327-159-2_4.
28. Qin S, Kim J, Arafat D, et al. Effect of normalization on statistical and biological interpretation of gene expression profiles. Front Genet 2012;3:160. DOI: 10.3389/fgene.2012.00160.
29. Lu J, Kerns RT, Peddada SD, et al. Principal component analysis-based filtering improves detection for Affymetrix gene expression arrays. Nucleic Acids Res 2011;39(13):e86. DOI: 10.1093/nar/gkr241.
30. Li J, Varghese RS, Ressom HW. RNA-seq data analysis. Methods Mol Biol 2024;2822:263–290. DOI: 10.1007/978-1-0716-3918-4_18.
31. van Lingen HJ, Suarez-Diez M, Saccenti E. Normalization of gene counts affects principal components-based exploratory analysis of RNA-sequencing data. Biochim Biophys Acta Gene Regul Mech 2024;1867(4):195058. DOI: 10.1016/j.bbagrm.2024.195058.
32. Kammoun A, Ravier P, Buttelli O. Impact of PCA pre-normalization methods on ground reaction force estimation accuracy. Sensors (Basel) 2024;24(4):1137. DOI: 10.3390/s24041137.
33. Daffertshofer A, Lamoth CJ, Meijer OG, et al. PCA in studying coordination and variability: A tutorial. Clin Biomech (Bristol) 2004;19(4):415–428. DOI: 10.1016/j.clinbiomech.2004.01.005.
34. Bjorklund M. Be careful with your principal components. Evolution 2019;73(10):2151–2158. DOI: 10.1111/evo.13835.
35. Townes FW, Irizarry RA. Quantile normalization of single-cell RNA-seq read counts without unique molecular identifiers. Genome Biol 2020;21(1):160. DOI: 10.1186/s13059-020-02078-0.
36. Skubleny D, Ghosh S, Spratlin J, et al. Feature-specific quantile normalization and feature-specific mean-variance normalization deliver robust bi-directional classification and feature selection performance between microarray and RNAseq data. BMC Bioinformatics 2024;25(1):136. DOI: 10.1186/s12859-024-05759-w.
37. Engreitz JM, Daigle BJ, Jr., Marshall JJ, et al. Independent component analysis: Mining microarray data for fundamental human gene expression modules. J Biomed Inform 2010;43(6):932–944. DOI: 10.1016/j.jbi.2010.07.001.
38. Kenn M, Cacsire Castillo-Tong D, Singer CF, et al. Microarray normalization revisited for reproducible breast cancer biomarkers. Biomed Res Int 2020;2020:1363827. DOI: 10.1155/2020/1363827.
39. Sahlabadi A, Chandren Muniyandi R, Sahlabadi M, et al. Framework for parallel preprocessing of microarray data using hadoop. Adv Bioinformatics 2018;2018:9391635. DOI: 10.1155/2018/9391635.
40. Piccolo SR, Sun Y, Campbell JD, et al. A single-sample microarray normalization method to facilitate personalized-medicine workflows. Genomics 2012;100(6):337–344. DOI: 10.1016/j.ygeno.2012.08.003.
41. Yao F, Coquery J, Le Cao KA. Independent principal component analysis for biologically meaningful dimension reduction of large biological data sets. BMC Bioinformatics 2012;13:24. DOI: 10.1186/1471-2105-13-24.
42. Chen X, Zhang B, Wang T, et al. Robust principal component analysis for accurate outlier sample detection in RNA-Seq data. BMC Bioinformatics 2020;21(1):269. DOI: 10.1186/s12859-020-03608-0.
43. Tseng CY, Salguero JA, Breidenbach JD, et al. Evaluation of normalization strategies for mass spectrometry-based multi-omics datasets. Metabolomics 2025;21(4):98. DOI: 10.1007/s11306-025-02297-1.
44. Zhao Y, Wong L, Goh WWB. How to do quantile normalization correctly for gene expression data analyses. Sci Rep 2020;10(1):15534. DOI: 10.1038/s41598-020-72664-6.
45. David CC, Jacobs DJ. Principal component analysis: A method for determining the essential dynamics of proteins. Methods Mol Biol 2014;1084:193–226. DOI: 10.1007/978-1-62703-658-0_11.
46. Liu X, Li N, Liu S, et al. Normalization methods for the analysis of unbalanced transcriptome data: A review. Front Bioeng Biotechnol 2019;7:358. DOI: 10.3389/fbioe.2019.00358.
47. Maadooliat M, Huang JZ, Hu J. Integrating data transformation in principal components analysis. J Comput Graph Stat 2015;24(1):84–103. DOI: 10.1080/10618600.2014.891461.
48. Feng C, Wang H, Lu N, et al. Log-transformation and its implications for data analysis. Shanghai Arch Psychiatry 2014;26(2):105–109. DOI: 10.3969/j.issn.1002-0829.2014.02.009.
49. West RM. Best practice in statistics: The use of log transformation. Ann Clin Biochem 2022;59(3):162–165. DOI: 10.1177/00045632211050531.
50. Moghadam N, Hegazy R. Data-centric AI for EEG-based emotion recognition: Noise filtering and augmentation strategies. Bioengineering (Basel) 2025;12(11). DOI: 10.3390/bioengineering12111264.
51. Zhao Y, Wang B, Mostofsky SH, et al. Covariate assisted principal regression for covariance matrix outcomes. Biostatistics 2021;22(3):629–645. DOI: 10.1093/biostatistics/kxz057.

52. Liu Z, Song R, Zeng D, et al. Principal components adjusted variable screening. Comput Stat Data Anal 2017;110:134–144. DOI: 10.1016/j.csda.2016.12.015.
53. Frost HR. Eigenvectors from Eigenvalues Sparse Principal Component Analysis (EESPCA). J Comput Graph Stat 2022;31(2):486–501. DOI: 10.1080/10618600.2021.1987254.
54. Elhaik E. Principal Component Analyses (PCA)-based findings in population genetic studies are highly biased and must be reevaluated. Sci Rep 2022;12(1):14683. DOI: 10.1038/s41598-022-14395-4.
55. Johnstone IM, Paul D. PCA in high dimensions: An orientation. Proc IEEE Inst Electr Electron Eng 2018;106(8):1277–1292. DOI: 10.1109/JPROC.2018.2846730.
56. Meng C, Zeleznik OA, Thallinger GG, et al. Dimension reduction techniques for the integrative analysis of multi-omics data. Brief Bioinform 2016;17(4):628–641. DOI: 10.1093/bib/bbv108.
57. Lee S, Zou F, Wright FA. Convergence and prediction of principal component scores in high-dimensional settings. Ann Stat 2010;38(6):3605–3629. DOI: 10.1214/10-AOS821.
58. Zhou HJ, Li L, Li Y, et al. PCA outperforms popular hidden variable inference methods for molecular QTL mapping. Genome Biol 2022;23(1):210. DOI: 10.1186/s13059-022-02761-4.
59. Cottrell S, Hozumi Y, Wei GW. K-nearest-neighbors induced topological PCA for single cell RNA-sequence data analysis. ArXiv 2023. PMID: 37961744.

REVIEW ARTICLE

Impact of the Ukrainian Conflict on Mothers and Young Infants Who have had to Migrate to Poland

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Received on: 09 December 2025; *Accepted on*: 18 February 2026; *Published on*: 08 April 2026

Abstract

The war in Ukraine has led to a major dislocation of the population into neighboring Poland. The conflict began in February 2014 with Russia's annexation of Crimea and escalated dramatically on February 24, 2022, when Russia launched a full-scale invasion of Ukraine. There has been widespread destruction, displacement, and humanitarian crises across the country. Millions of Ukrainians have fled their homes; over 2 million people had to flee Ukraine within 2 weeks and there have been more than 9.4 million border crossings. In this article, we have focused on the social impact of population migration, with particular attention to its effects on perinatal outcomes. Broad estimates suggest that around 2,65,000 Ukrainian women were pregnant when the war began and approximately 80,000 were expected to give birth in the first 3 months of the conflict. The Ukrainian conflict significantly affected the care of pregnant mothers—many experienced suboptimal antenatal care, underwent high levels of stress, and saw considerable uncertainty during displacement. Their infants had high needs for medical care. The arrival of large numbers of Ukrainians has led to noticeable changes in Poland's social landscape. The Polish government and society have responded to population movements from Ukraine by offering all-possible protection and wide-ranging support. Poland has received global recognition for its efforts to support Ukrainian mothers and children during the refugee crisis.

Keywords: Crimea, Donetsk, Humanitarian crises, Kharkiv, Kherson, Luhansk, Newborn, Population dislocation, Russians, Zaporizhzhia.

Newborn (2026): 10.5005/jp-journals-11002-0151

Key Points

- The war in Ukraine has led to a major dislocation of the population into neighboring Poland. This population movements from Ukraine to Poland can be ascribed to geographic proximity, a long-shared border, and cultural and linguistic similarities.
- In this article, we have focused on the social impact of population migration, with particular attention to its effects on perinatal outcomes. The large-scale movement of people has placed significant pressure on healthcare systems, social services, and community support networks.
- The exact figures for the number of pregnant Ukrainian women who migrated to Poland are not readily available. Broad estimates suggest that around 265,000 Ukrainian women were pregnant when the war began and approximately 80,000 were expected to give birth in the first 3 months of the conflict.
- The Ukrainian conflict has significantly affected the care of pregnant mothers. Many pregnant women experienced disrupted antenatal care, high levels of stress, and uncertainty during displacement, increasing risks for both maternal and fetal health.
- The arrival of large numbers of Ukrainians has led to noticeable changes in Poland's social landscape. The Polish government and society responded to population movements from Ukraine by offering temporary protection and wide-ranging support. Poland has received global recognition for its efforts to support Ukrainian mothers and children during the refugee crisis.

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How to cite this article: Frydrysiak-Brzozowska A, Singh S, Adamczyk-Gruszka O, *et al*. Impact of the Ukrainian Conflict on Mothers and Young Infants Who have had to Migrate to Poland. Newborn 2026;5(1):26–38.

Source of support: Nil

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

Introduction

The war in Ukraine has led to a major dislocation of the population into neighboring Poland. The conflict began in February 2014 with Russia's annexation of Crimea and escalated dramatically on February 24, 2022, when Russia launched a full-scale invasion of Ukraine.[1] There has been widespread destruction, displacement, and humanitarian crises across the country. Millions of Ukrainians have fled their homes; over 2 million people had to flee Ukraine within 2 weeks and there have been more than 9.4 million border crossings (Fig. 1).[2] There are more than 1.5 million registered refugees, of which 90% are women, children, and elderly people; the primary reasons were to escape violence, destruction, and insecurity. The war has affected civilians, infrastructure, and essential services, creating urgent needs for food, shelter, healthcare, education, and protection.[3–5]

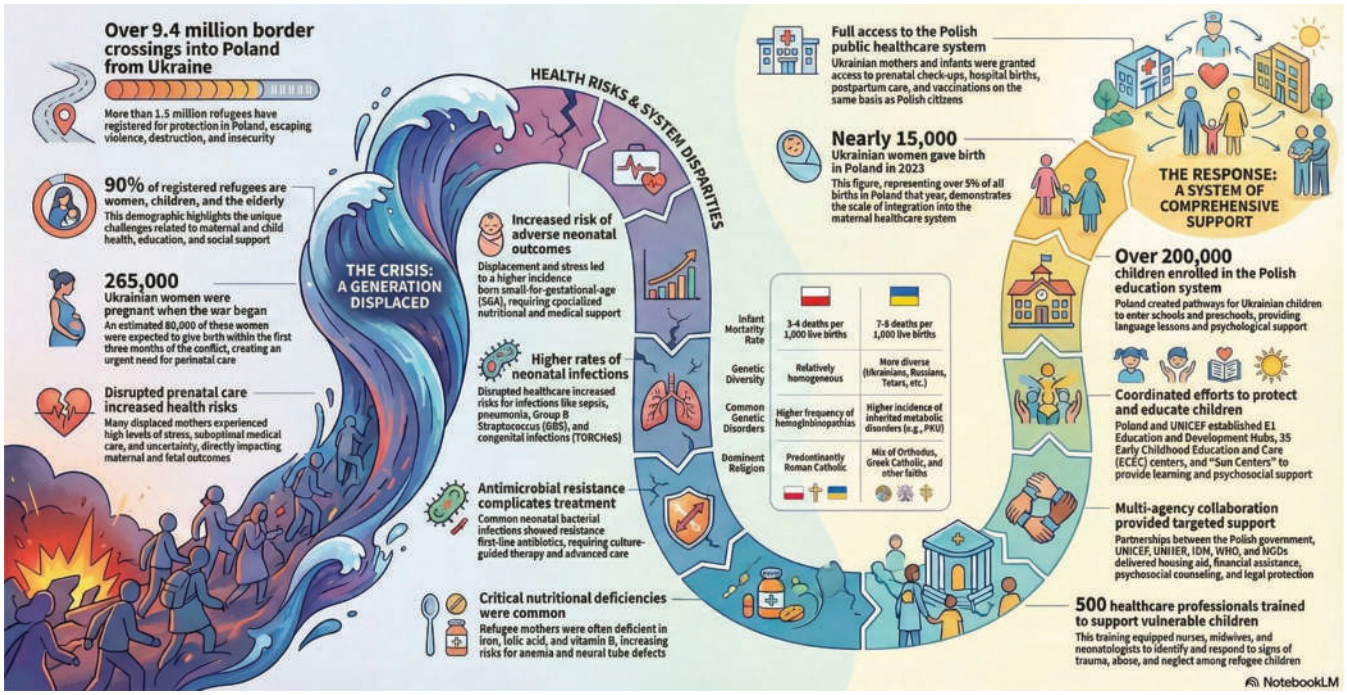

Fig. 1: Hope in displacement: Supporting Ukrainian mothers and infants in Poland. This figure has been prepared with NotebookLM (Google)

International organizations, governments, and humanitarian agencies have mobilized to provide emergency aid, support displaced populations, and respond to the social, economic, and health impacts of the conflict.

The population movements from Ukraine to Poland can be ascribed to geographic proximity, a long-shared border, and cultural and linguistic similarities. Poland is one of Ukraine's closest neighbors, allowing people to cross the border quickly and safely, often with minimal travel costs. The shared border made large-scale movement possible even in emergency conditions. In addition, similarities in language, history, and cultural traditions helped Ukrainians adapt more easily to life in Poland, reducing feelings of displacement. These factors, combined with existing Ukrainian communities in Poland, made Poland a natural and practical destination for many people leaving Ukraine. The migrants have come from multiple regions, particularly those most affected by conflict and insecurity. Many refugees originated from eastern and southern Ukraine, including areas such as Donetsk, Luhansk, Kharkiv, Zaporizhzhia, and Kherson, where military activity has been intense.[6–8] Large numbers also left central and northern regions, including Kyiv and surrounding oblasts, due to threats of attacks or occupation. People from western regions, which were initially less affected, sometimes moved as well to join family or seek economic opportunities. The flow from these diverse regions reflects both immediate safety concerns and longer-term considerations for work, education, and family reunification. Figure 2 shows emergency mass shelter facilities established in sports halls in Poland during the initial influx of refugees from Ukraine.

In this article, we have focused on the social impact of population migration, with particular attention to its effects on perinatal outcomes. The large-scale movement of people has placed significant pressure on healthcare systems, social services, and community support networks. Understanding these effects is essential for shaping effective health, social, and protection responses for migrant mothers and infants. Figure 3 shows the key factors influencing perinatal and neonatal outcomes among Ukrainian mothers and young infants displaced to Poland during the ongoing conflict.

Maternal Factors and Impact on Perinatal Outcomes

Migration of Pregnant Women

The exact figures for the number of pregnant Ukrainian women who migrated to Poland are not readily available. During the initial months after Russia's full-scale invasion in 2022, many of the refugees entering Poland were women of reproductive age, and a significant share were pregnant when they fled. Broad estimates suggest that around 2,65,000 Ukrainian women were pregnant when the war began and approximately 80,000 were expected to give birth in the first 3 months of the conflict.[9,10] All these women did not stay in Poland or were counted locally, and so the statistics may not be readily usable for healthcare planning. However, nearly 15,000 Ukrainian women gave birth in Poland in 2023, constituting more than 5% of all births in the country that year, showing that many pregnant refugees accessed maternal care after arriving.[11,12]

Care of Pregnant Mothers

The Ukrainian conflict has significantly affected the care of pregnant mothers, particularly those who were forced to flee their homes and seek safety in countries such as Poland. Many pregnant women experienced disrupted antenatal care, high levels of stress, and uncertainty during displacement, increasing risks for both maternal and fetal health. In Poland, pregnant Ukrainian women were granted access to prenatal services, including routine check-ups, ultrasounds, and hospital-based maternity care within the public health system. With support from United Nations Children's Fund (UNICEF), non-governmental organizations (NGOs), and

Fig. 2: Emergency mass shelter facilities established in sports halls in Poland during the initial influx of refugees from Ukraine (2022). Large-capacity gymnasiums and sports arenas were rapidly repurposed to accommodate displaced families, with rows of temporary camp beds and basic bedding arranged to provide short-term shelter and essential support services
Sources: Hall at ul. Sowińskiego, Szczecin (Gazeta Wyborcza Szczecin); Hall at ul. Dożynkowa, Poznań (Poznań University of Economics and Business); Wrocław (Gazeta Wrocławska)

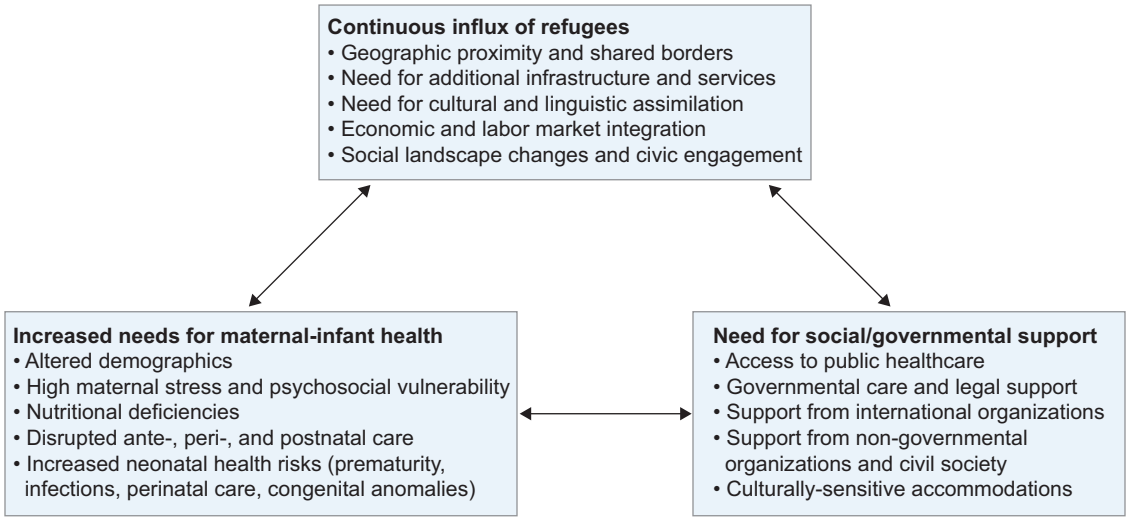

Fig. 3: Key factors influencing perinatal and neonatal outcomes among Ukrainian mothers and young infants displaced to Poland during the ongoing conflict

local authorities, services such as psychosocial support, health education, and language assistance were provided to help ensure safe pregnancies and improve maternal outcomes.[13]

The Ukrainian conflict increased the average maternal age among displaced pregnant women. Women who were already pregnant or who chose to continue planned pregnancies during displacement were often in their late twenties or thirties, reflecting pre-existing trends of delayed childbearing in Ukraine. Younger women were more likely to postpone pregnancy due to insecurity, separation from partners, and economic uncertainty, while older women were more likely to proceed with pregnancy despite the conflict.[14] As a result, healthcare providers in host countries observed a higher proportion of pregnancies among older mothers, with implications for prenatal monitoring and maternal care needs.

Maternal Health Factors

Polish and Ukrainian societies differ in several ethnic and cultural aspects. Poland is ethnically more homogeneous with most identifying themselves as ethnic Poles; Ukraine is more diverse, with significant populations of Russians, Belarusians, Crimean Tatars, and other minority groups. Language is another key difference: Polish, a West Slavic language, dominates in Poland. In Ukraine, most people speak Ukrainian, an East Slavic language, and some use Russian. Religious composition also varies; Poland is predominantly Roman Catholic. On the other hand, Ukraine has a mix of Orthodox, Greek Catholic, and other faiths.

The health statistics show considerable differences. The infant mortality rates (IMRs) are quite dissimilar between Poland and Ukraine, reflecting variations in healthcare systems and living conditions. In Poland, the IMR has been about 3–4 deaths per 1,000 live births, thanks to well-developed prenatal and neonatal care, widespread vaccination, and effective public health programs.[15] In contrast, Ukraine has had a higher IMR of around 7–8 deaths per 1,000 live births, with some regions experiencing even greater challenges due to limited healthcare access, economic constraints, and the impact of conflict.[16,17] These differences underscore the additional health risks faced by displaced Ukrainian infants in Poland, who may arrive with complications linked to disrupted prenatal care and stressful living conditions.

Poland and Ukraine differ in their levels of genetic diversity due to historical migration patterns, population size, and ethnic composition. Poland is relatively homogeneous, with the majority of the population being ethnic Poles with limited genetic variation. Ukraine, in contrast, is more genetically diverse, reflecting its larger number of ethnic groups; the society is comprised of several subgroups including Ukrainians, Russians, Belarusians, Crimean Tatars, and other minorities. There is also a history of migrations and interactions with neighboring regions. This greater genetic diversity in Ukraine can influence susceptibility to certain inherited conditions, disease prevalence, and responses to medical interventions, whereas Poland's more homogeneous population may exhibit more uniform genetic traits across the majority population.[18]

In both Poland and Ukraine, several genetic disorders are observed with varying frequency, influenced by population genetics, ethnic composition, and healthcare screening practices. Cystic fibrosis, congenital hypothyroidism, and phenylketonuria (PKU) are seen in both countries. Hemoglobinopathies such as beta-thalassemia, sickle cell disease, and other abnormal hemoglobin variants are seen more frequently in Poland. Ukraine has a higher incidence of inherited metabolic disorders such as phenylketonuria, galactosemia, and various amino acid, organic acid, and fatty acid oxidation disorders.[19] Both countries have implemented neonatal screening programs to detect these conditions early, enabling timely interventions and improving long-term health outcomes for affected infants.

Socioeconomic Needs

The demographics of Ukrainian pregnant mothers who arrived in Poland reflect a wide range of ages, educational backgrounds, and family circumstances; most women of reproductive age are typically in their late-twenties to mid-thirties. Many were married or partnered, but a significant proportion were single or separated from family due to the conflict. Poland has provided broad acceptance and integration for these women, granting access to public healthcare, prenatal services, and social support on the same basis as Polish citizens. Combined with assistance from NGOs and international organizations like UNICEF, this inclusive approach has helped ensure that Ukrainian pregnant mothers receive medical care, psychosocial support, and practical resources despite the challenges of displacement.

The Ukrainian conflict has heightened the vulnerability of pregnant mothers, particularly single mothers, who often arrived in Poland without family or partner support. Separation from relatives, the stress of displacement, and limited social networks increased the emotional and practical challenges of pregnancy, including childcare, healthcare access, and daily living needs. To address these gaps, Polish authorities, NGOs, and international organizations such as UNICEF provided targeted support, including counseling, social services, peer networks, and community programs to assist single mothers. These measures helped reduce isolation, ensured access to healthcare and social protection, and offered essential guidance for managing pregnancy under the difficult circumstances of displacement.

Ukrainian mothers in Poland often face difficulties such as limited access to transportation needed to reach medical centers. During displacement, many women faced long journeys, border crossings, and limited availability of safe or affordable transport, which delayed or disrupted antenatal care.[20] In Poland, newly-arrived pregnant women often struggled with unfamiliar transport systems, language barriers, and mobility constraints, especially in the later stages of pregnancy. To address these challenges, local authorities, NGOs, and international organizations helped arrange transportation to hospitals and clinics, provided information on public transport, and offered outreach services closer to refugee communities. Improving transport access was essential to ensuring timely prenatal check-ups, safe deliveries, and emergency obstetric care.

Nutritional Needs

Refugee mothers were at increased risk of specific nutritional deficiencies due to displacement, stress, and disrupted access to adequate food during pregnancy. Common deficiencies included iron, folic acid, and vitamin D, which are critical for maternal health and fetal development. In response, Polish healthcare providers, in coordination with NGOs and UNICEF, implemented programs to assess nutritional status, provide supplementation, and offer dietary counseling tailored to the needs of pregnant Ukrainian women.[21,22] These interventions helped reduce risks such as anemia, neural tube defects, and poor fetal growth, supporting healthier pregnancy outcomes despite the challenges posed by conflict and displacement.

Economic Needs

Ukrainian pregnant mothers have received targeted economic support in Poland to help meet the costs associated with displacement and pregnancy. The Polish government, in coordination with local authorities and NGOs, provided financial assistance, including cash benefits, maternity allowances, and support for housing and essential needs.[23] Additional support from international organizations like UNICEF helped cover healthcare-related expenses, transport to medical facilities, and access to prenatal services. These measures have been critical in reducing financial stress, enabling pregnant mothers to access proper care, and ensuring the health and well-being of both mothers and their unborn children during the challenging circumstances of displacement.

Cultural Preferences

Immigrant mothers from Ukraine brought with them diverse religious beliefs that sometimes influenced their preferences for medical care.[3] Most Ukrainian refugees identify as Orthodox or Greek Catholic, and others follow Roman Catholicism, Protestantism, or are non-religious. These beliefs affected decisions around prenatal and perinatal care, childbirth practices, and acceptance of certain medical interventions. Polish healthcare providers, supported by NGOs and organizations like UNICEF, worked to ensure culturally and religiously sensitive care, offering respectful communication, accommodation of spiritual needs, and guidance aligned with both medical standards and mothers' values. This approach helped build trust, improve healthcare access, and support positive maternal and neonatal outcomes among displaced Ukrainian mothers.

Some of these Ukrainian mothers expressed a preference regarding the religion of their healthcare providers, often seeking caregivers who shared their Orthodox, Greek Catholic, or Roman Catholic faith.[24] A very small proportion expressed avoidance of Jewish healthcare providers, reflecting personal, cultural, or historical sensitivities. Mostly, preferences were influenced not by religion but by comfort, trust, and the desire for culturally and spiritually sensitive care. Polish healthcare facilities, in collaboration with NGOs and organizations like UNICEF, have worked to accommodate these preferences where possible, while ensuring that all medical decisions remain guided by professional standards. By respecting maternal considerations alongside evidence-based care, providers were able to foster trust, reduce stress, and improve maternal and neonatal outcomes among displaced Ukrainian families.

Among Ukrainian mothers displaced to Poland, cultural and personal preferences regarding fetal gender have occasionally influenced pregnancy expectations, though such preferences vary widely by family and region. While most mothers prioritize the health and safety of the child above all, some may express a preference for a particular gender due to family composition, cultural traditions, or personal reasons. Polish healthcare providers, together with NGOs and organizations like UNICEF, have respected these preferences while emphasizing unbiased medical care, ensuring that fetal monitoring, prenatal support, and counseling remain focused on the well-being of both mother and child. By combining sensitivity to personal or cultural expectations with professional care standards, healthcare teams have supported positive outcomes for Ukrainian mothers and their newborns.

Risk of Prenatal Infections

The Ukrainian conflict has increased the risk of prenatal infections among pregnant mothers, largely due to disrupted healthcare services, delayed antenatal visits, and challenging living conditions during displacement.[25] Interruptions in routine screening and treatment for infections such as hepatitis, human immunodeficiency virus (HIV), syphilis, and other preventable conditions posed risks to both maternal and fetal health.[26] In host countries like Poland, pregnant Ukrainian women were integrated into prenatal care systems, where screening, treatment, and preventive services were provided according to national guidelines.[27] Support from international organizations and NGOs helped strengthen early detection, continuity of care, and health education, reducing the potential impact of prenatal infections on pregnancy and neonatal outcomes.

Medical Terminations of Pregnancy

Reliable, detailed figures on the number of Ukrainian medical terminations of pregnancy for medical vs other reasons during the conflict period are not systematically reported in publicly available official data. However, national health statistics from Ukraine during the full-scale invasion period provide some context. According to the Ukrainian Ministry of Health's Public Health Center, Ukrainian women underwent approximately 90,000 abortions over the first 2 years of the full-scale invasion (2022–2023), based on registered procedures. This figure includes both spontaneous pregnancy losses (miscarriages) and medically-performed terminations, whether for health indications or other reasons.[28] These numbers total represents a decline compared with prewar years but reflects ongoing reproductive health needs amid war disruptions. In 2023, there were about 45,000 abortions were recorded in Ukraine, down slightly from 2022, though experts caution that official data may undercount procedures due to population displacement, disrupted health services in conflict zones, and unregistered or out of country procedures not captured in Ukrainian statistics.[29] These statistics do not break down terminations by specific medical indication, and there is no comprehensive international dataset that isolates conflict related medical terminations (such as those due to fetal anomaly or maternal health risk) separately from routine reproductive care. The available totals reflect all legally recorded terminations and spontaneous losses during the war affected years rather than a distinct count of conflict driven medical terminations.

Number of Ukrainian Women Who Gave Birth in Poland

In 2023, nearly 15,000 Ukrainian women gave birth in Poland, making Ukrainian births a significant portion of all live births that year.[12,30] These figures reflect births after arrival rather than the specific count of neonates transported into Poland immediately after birth, they show that a substantial number of Ukrainian infants have been part of the refugee flows and have entered the Polish healthcare and protection systems as newborns or young children.

Maternal Postpartum Care

The Ukrainian conflict has had a notable impact on maternal postpartum care in Poland, as many displaced mothers arrived shortly before or after giving birth. Disruption, stress, and separation from family support networks increased the need for comprehensive postpartum services, including medical follow-up, mental health support, and guidance on infant care.[31] Poland's healthcare system provided Ukrainian mothers with access to postpartum check-ups, breastfeeding support, and neonatal care on the same basis as Polish citizens. With additional support from UNICEF and NGOs, services such as psychosocial counseling, parenting education, and community-based support were strengthened to address the complex physical and emotional needs of mothers during the postpartum period.

Impact of Perinatal Factors on Neonatal Outcomes

Need for Neonatal Transportation to Better-equipped Hospitals

There are no comprehensive official figures specifically on the number of Ukrainian neonates transported into Poland as

newborns, but related data on births to Ukrainian refugee mothers in Poland provides a useful context. From the start of the war in February 2022 through the first part of the refugee response, thousands of Ukrainian infants were born in Polish hospitals after their mothers arrived as refugees. For example, between February 24, 2022 and August 31, 2022, approximately 4,537 newborns were reported born in Poland to Ukrainian mothers, and other estimates put the number of Ukrainian newborns born in Polish hospitals in that period at several thousand.[12]

Needs for Neonatal Intensive Care

Most publicly available transport statistics focus broadly on medical evacuations or refugee flows. Data on medical evacuation programs, such as Ukraine's MedEvac initiative have reported that over 5,000 patients were transported abroad for specialized care but these figures are not broken down by specific medical conditions.[32,33] This initiative is a humanitarian medical evacuation program designed to provide urgent care to critically-ill patients who cannot access necessary treatment locally due to the conflict. Individual cases of highly vulnerable infants being moved for urgent treatment have been reported but no comprehensive official statistics are available about transports for serious conditions such as prematurity, birth asphyxia, sepsis, or congenital anomalies. Some individual cases of infants with complex multisystem congenital conditions being evacuated have been reported as part of humanitarian and medical coordination efforts, but these reports still remain anecdotal and have not yet been formally counted.

The Ukrainian conflict has affected the incidence of infants born small-for-gestational age (SGA) among the refugee populations. Displacement, stress, disrupted prenatal care, and limited access to adequate nutrition and medical monitoring during pregnancy increased the risk of growth restriction *in utero*.[34,35] Upon arrival in Poland, many infants were assessed through neonatal and pediatric services, where early detection of SGA allowed for timely interventions, including nutritional support and monitoring for potential complications. Support from Polish healthcare providers, UNICEF, and NGOs has been essential in addressing these risks, ensuring that affected infants receive appropriate care to promote healthy growth and development despite the challenges of displacement.[13]

Neonatal Infections

Among Ukrainian infants, especially those displaced to Poland, certain neonatal infections have been frequently observed due to disrupted prenatal care, stressful living conditions, and limited early healthcare access. Common infections include sepsis and bloodstream infections, respiratory infections such as pneumonia, gastrointestinal infections, skin, and soft tissue infections.

Group B streptococcus (GBS) has been identified as a significant cause of neonatal infections among Ukrainian infants, particularly in those born prematurely or with low birth weight.[36–39] Group B streptococcus can lead to serious conditions such as sepsis, pneumonia, and meningitis, contributing to morbidity and mortality in newborns. Although exact country-specific statistics for Ukrainian infants during the conflict are limited, studies from similar Eastern European populations suggest that GBS is one of the leading bacterial pathogens in neonatal infections, accounting for a substantial proportion of early-onset sepsis cases. In Poland, healthcare providers caring for displaced Ukrainian infants have prioritized GBS screening, early antibiotic treatment, and preventive protocols to reduce the risk and impact of these infections.

Neonatal bacterial infections among Ukrainian infants have shown concerning levels of antimicrobial resistance, complicating treatment and increasing the risk of severe outcomes. Common pathogens such as *Escherichia coli*, *Klebsiella* species, and *Staphylococcus aureus*, including methicillin-resistant staphylococcus aureus (MRSA) strains, have been reported with resistance to first-line antibiotics. Contributing factors include disrupted healthcare systems, limited access to timely prenatal and neonatal care, and prior inappropriate or incomplete antibiotic use during displacement. In Poland, clinicians caring for displaced Ukrainian neonates have emphasized culture-guided therapy, infection control measures, and close monitoring, often in collaboration with pediatric infectious disease specialists, to effectively manage resistant infections while minimizing the spread of drug-resistant bacteria.

Some infants may need to tested for congenital infections such as the Toxoplasmosis, Rubella, Cytomegalovirus, Herpes, and Syphilis (TORCHeS). Premature and low-birth-weight infants are particularly vulnerable.[40,41] Polish healthcare providers, with support from UNICEF and NGOs, have focused on early screening, timely treatment, and preventive measures such as hygiene promotion, breastfeeding support, and vaccination to reduce the incidence and complications of these infections.

The medical needs of premature Ukrainian infants in Poland often differ from those of locally-born Polish preterm infants due to the effects of displacement and disrupted prenatal care. Many Ukrainian infants experienced limited or interrupted maternal healthcare, higher rates of maternal malnutrition, and exposure to stress or infections during pregnancy, increasing the risk of low birth weight, growth restriction, and neonatal complications. They may also have incomplete vaccination and higher susceptibility to sepsis or other infections.[42] As a result, Ukrainian premature infants frequently require additional screening, infection prevention, nutritional support, and specialized follow-up care compared with Polish infants, even though both groups share core needs such as respiratory support, thermoregulation, and monitoring for prematurity-related complications.

Congenital Anomalies

The neonatal congenital anomalies such as cardiac defects, neural tube defects, cleft lip and palate, and musculoskeletal malformations are broadly similar between both countries. Poland has established a comprehensive prenatal screening and neonatal diagnostic programs, enabling early detection through ultrasound, genetic testing, and newborn examinations. Early identification allows timely medical or surgical interventions, counseling, and long-term management, which significantly improve outcomes for affected infants. Public health initiatives, including folic acid supplementation and maternal care programs, have also contributed to reducing the incidence of certain preventable congenital anomalies in the population.

Primary Care for Infants

The Ukrainian conflict has significantly affected access to primary care for infants in Poland, as large numbers of displaced families entered the country with urgent health needs. Many Ukrainian infants required immediate registration with primary healthcare providers to access routine check-ups, vaccinations, and developmental monitoring. Poland integrated refugee children

into its public healthcare system, granting them access to primary care on the same basis as Polish citizens. With support from UNICEF and NGOs, primary care services were expanded through outreach, language assistance, and training for healthcare workers. These measures helped ensure continuity of care for infants, despite the increased demand on Poland's healthcare system.

Neonatal Vaccine Coverage

The Ukrainian conflict has had a significant impact on vaccination coverage for infants and young families in Poland. Many Ukrainian children arrived with disrupted or incomplete immunization schedules due to the breakdown of healthcare services during the war and displacement. In response, Polish health authorities, in collaboration with UNICEF and other partners, integrated refugee children into the national vaccination program, providing free access to routine and catch-up immunizations.[13] Outreach campaigns, information in Ukrainian, and support from local health centers helped parents understand vaccination schedules and overcome barriers such as language and system unfamiliarity.[43] These efforts were essential in protecting infants and young children from vaccine-preventable diseases and maintaining public health in host communities.

Young Ukrainian Families have Needed Considerable Social Support

Many young migrant families need support. Under temporary protection arrangements, they were allowed to stay legally in the country without applying for traditional asylum. They were given rights to work, access healthcare, enroll children in schools, and receive social assistance. Registration systems helped Ukrainians obtain identification numbers, which were essential for accessing services and employment. These protections reduced vulnerability and helped displaced people achieve a level of stability and safety while living in Poland.

Ukrainians who moved to Poland were provided with access to public services and community support (Fig. 4). They were entitled to healthcare on the same basis as Polish citizens, including emergency treatment, primary care, and specialist services. Psychological support was also offered to help people cope with trauma caused by war and displacement. In addition, social care services supported vulnerable groups. Alongside state services, non-governmental organizations and volunteers played an important role in providing everyday care, guidance, and assistance to Ukrainians adjusting to life in Poland.

Fig. 4: Reception, coordination, and community support services for displaced Ukrainian families in Poland (2022). (Top left) Crowded railway station concourse adapted to receive newly arrived refugees, with volunteer registration and information desks; (Top right) On-site assistance point displaying "Solidarni z Ukrainą" ("Solidarity with Ukraine") signage, providing guidance, transportation coordination, and humanitarian information; (Bottom left) Designated child-friendly space within a reception facility offering supervised play and psychosocial support; (Bottom right) Volunteer-run donation and distribution center organizing clothing, toys, and essential supplies for displaced families. These images illustrate the rapid mobilization of civil society, municipal authorities, and humanitarian organizations to provide immediate reception, protection, and basic services during the acute phase of displacement

Sources: Hall at ul. Sowińskiego, Szczecin (Gazeta Wyborcza Szczecin); Hall at ul. Dożynkowa, Poznań (Poznań University of Economics and Business); Wrocław (Gazeta Wrocławska)

Young siblings in these families were given access to formal education within the Polish school system. Poland has developed pathways for more than 2,00,000 Ukrainian children to enroll in formal schools and preschools.[44–46] These pathways include preparatory classes, Polish language lessons, and support from teaching assistants to help children adapt to the curriculum.[13] Schools have also offered psychological and social support to address the emotional impact of displacement. By creating flexible and inclusive education programs, Poland has ensured that Ukrainian children can continue their learning, maintain a sense of normalcy, and integrate more easily into Polish society. Nearly 2,60,000 children have been supported with specialized early childhood development, Polish language learning or digital learning in dedicated hubs for children have helped, including children with disabilities. Nearly 5,000 Polish teachers and assistants have been trained. About 17,000 digital tablets and 1,400 computers have been provided to bridge learning gaps. Over 3,50,000 children in 2,220 education institutions, including schools, community centers, daycares, and preschools, have been supported with learning materials. These investments in the Polish system have been provided to whole classrooms, schools, and preschools, meaning Polish children have also benefitted in addition to their Ukrainian peers. Universities also opened opportunities for Ukrainian students by offering fee waivers, scholarships, and flexible admission procedures. These measures aimed to reduce disruption to education and support the long-term integration of Ukrainian learners in Poland.

Housing support was provided through both government programs and community initiatives. At the beginning of the crisis, many were accommodated in reception centers, sports halls, and temporary shelters. The Polish government later introduced financial assistance for host families and subsidies to help Ukrainians rent private accommodation. Local authorities worked to match refugees with available housing, while charities and volunteers helped find longer-term solutions. Although housing shortages remained a challenge, this support helped many Ukrainians secure safe and stable places to live in Poland.

Polish courts have appointed temporary guardians for unaccompanied Ukrainian children to ensure their safety and well-being. These guardians, often volunteers, or social workers, were legally responsible for protecting the children's rights, making decisions about their care, and helping them access education, healthcare, and social services. This system provided essential oversight for children who arrived without parents or adult relatives, preventing neglect or exploitation. By assigning temporary guardians, Poland aimed to offer a stable and supportive environment for vulnerable young refugees while longer-term solutions were arranged.[47]

Impact on Polish Social and Economic Needs

The arrival of large numbers of Ukrainians has led to noticeable changes in Poland's social landscape. Ukrainian communities now form an important component of Polish cities and towns, influencing everyday life through language, culture, and traditions. Schools, workplaces, and public services have become more diverse, requiring greater emphasis on inclusion and integration. At the same time, Polish society has seen strong civic engagement, with volunteers and organizations working to support newcomers. While challenges such as housing pressure and service demand have emerged, the increased diversity has also strengthened social ties and cultural exchange within Poland.

The arrival of large numbers of Ukrainians has changed Poland's economic landscape in several ways. Many Ukrainians have entered the labor market, helping to address labor shortages in sectors such as construction, manufacturing, healthcare, and services. Their participation has increased overall workforce supply and contributed to economic activity and tax revenues. At the same time, government spending on social support, housing, and public services has risen, creating short-term financial pressure. In the longer term, the integration of Ukrainians into the workforce has the potential to support economic growth and strengthen Poland's economy.[48,49]

The influx of Ukrainian people has placed considerable pressure on Poland's public services and infrastructure. Hospitals, schools, housing systems, and transportation networks now face increased demands, requiring rapid adaptation and expansion. Local governments have had to allocate additional funds and resources to meet the needs of refugees, while social services and NGOs worked to provide immediate support. The sudden population rise also created challenges in employment, language access, and community integration. Despite these pressures, Poland has managed a large-scale humanitarian response, balancing urgent needs with longer-term planning with some success.

Movement of Polish Immigrants Back to Ukraine or to Other Schengen Areas

Some Ukrainians who moved to Poland have since returned to Ukraine or relocated to other European countries. As conditions in certain parts of Ukraine stabilized, some people chose to go back to reunite with family or rebuild their lives. Others moved on to other Schengen countries such as Germany, Czechia, or Italy in search of better job opportunities, long-term housing, or family connections. Changes in personal circumstances, employment prospects, and levels of support across Europe have influenced these decisions, leading to a more fluid pattern of movement rather than permanent settlement in one country.

The Polish government and society responded to population movements from Ukraine by offering temporary protection and wide-ranging support. Ukrainians arriving in Poland were granted legal status that allowed them to stay, work, and access public services. The government provided access to healthcare, education for children, and social benefits, while local authorities helped with accommodation and registration. At the same time, Polish citizens, charities, and volunteer groups played a major role by offering housing, food, transportation, and donations. This combined state and community response helped millions of Ukrainians settle more safely and quickly in Poland during the crisis.

Poland has worked closely with UNICEF to support Ukrainian children affected by displacement. Together, they have focused on ensuring access to education, healthcare, and psychosocial support for young refugees. United Nations Children's Fund has helped provide learning materials, safe spaces, and programs to address trauma and emotional well-being. Coordination with Polish authorities has also strengthened child protection systems, including assistance for unaccompanied and vulnerable children. This collaboration has been essential in helping Ukrainian children adapt to life in Poland and continue their development despite the disruption caused by conflict. Around 1,38,000 aid packages have been provided to individuals and families through our joint work with municipalities and local NGOs.[13,50]

Globally-coordinated Efforts

Poland has received global recognition for its efforts to support Ukrainian mothers and children during the refugee crisis. The country's comprehensive response, including access to healthcare, education, housing, and social services, has been recognized by international organizations and governments. Poland's policies ensured that mothers and children could find safety, maintain their well-being, and continue their daily lives despite displacement. Collaborative initiatives with organizations such as the UNICEF and local NGOs have further strengthened this support.

United Nations Children's Fund has helped the Polish government and NGOs to establish 61 Education and Development Hubs across the country. These hubs provide Ukrainian children with access to formal and non-formal education, language support, and psychosocial services.[13,51] They serve as safe spaces where children can continue learning, participate in creative and recreational activities, and receive guidance to help them integrate into Polish schools. By combining government resources, NGO expertise, and UNICEF support, these hubs have helped displaced children maintain their education and well-being during the crisis.

Early Childhood Education and Care (ECEC) centers have helped support Ukrainian children affected by displacement. At present there are 35 such centers; these provide preschool-aged children with safe learning environments, early education programs, and opportunities for social interaction.[13,51] They also offer language support, psychosocial care, and activities that help children cope with the stress of displacement. By expanding early childhood services, Poland has helped ensure that young Ukrainian children can continue their development, build essential skills, and adapt more smoothly to life in a new country.

Poland has developed several models to support Ukrainian pregnant mothers, focusing on education, development, therapy, and psychosocial well-being. These programs provide prenatal care, guidance on maternal and child health, and access to medical services. They also offer psychosocial support, including counseling, stress management, and peer support groups, to help mothers cope with the challenges of displacement. Educational workshops and parenting programs help expectant mothers prepare for childbirth and early child-rearing in a new environment. By combining health, learning, and emotional support, these initiatives aim to protect the well-being of both mothers and their children.

United Nations Children's Fund-supported Sun Centers currently support about 10,000 children in Krakow. These provide safe and welcoming spaces for Ukrainian children and families displaced by the conflict. These centers offer educational activities, psychosocial support, and recreational programs that help children cope with trauma and maintain a sense of normalcy.[50] They also provide guidance and resources for parents, including information on healthcare, schooling, and social services. By combining learning, play, and emotional support, the Sun Centers help Ukrainian families adjust to life in Poland while promoting children's well-being and development.

Efforts of the Polish Government

Ukrainian children escaping conflict are particularly vulnerable to violence, exploitation, and abuse, making specialized care essential. In response, Poland has trained around 500 healthcare professionals—including nurses, midwives, and neonatologists—across the country. This training equips medical staff to identify and respond to signs of trauma, abuse, or neglect, while providing age-appropriate healthcare and psychosocial support. By strengthening the skills of healthcare workers, Poland aims to protect displaced children, ensure their well-being, and provide safe, informed care during a highly vulnerable time.

The Polish government worked closely with municipalities in areas with high concentrations of refugees to reach out to the Ukrainian population. Local authorities helped coordinate registration, access to healthcare, education, housing, and social services, ensuring that displaced families received timely assistance. Municipalities also collaborated with NGOs, volunteers, and international organizations to provide information, support, and resources, helping refugees integrate into local communities and access essential services efficiently.

In spring–summer 2022, the Polish government and UNICEF reached out to more than 5,00,000 Ukrainian refugees to promote immunization, child health, and overall well-being. Working in partnership with local municipalities and NGOs, they provided information on vaccination schedules for poliomyelitis and hepatitis A, access to healthcare services, and guidance on preventive care. These efforts helped ensure that displaced children received necessary immunizations, regular check-ups, and medical support despite the disruption caused by conflict. By combining government resources, UNICEF expertise, and community networks, Poland strengthened public health protection for a large refugee population.[50]

Polish governmental bodies, institutions, local administrations, and grassroots civil society organizations have collaborated with the UN to strengthen policies and systems supporting Ukrainian mothers and infants. This partnership focused on improving access to healthcare, education, psychosocial services, and social protection for displaced families. Efforts included developing care programs, training healthcare professionals, and creating safe spaces for mothers and children. These activities were particularly prominent in major cities such as Krakow, Warsaw, and Gdansk, where large numbers of refugees arrived, ensuring coordinated support and effective delivery of essential services.

The Polish Ministry of Family and Social Policy and the Ministry of Education and Science (MoES) collaborated with UNICEF to ensure the protection, education, and health of Ukrainian mothers and infant refugees.[52,53] This partnership focused on providing access to healthcare services, early childhood education, and psychosocial support, as well as safeguarding children from exploitation and abuse. By coordinating policies, programs, and resources, the ministries and UNICEF strengthened systems to meet the specific needs of displaced families, ensuring that mothers and infants could receive comprehensive care and support during their time in Poland.

Poland worked jointly with United Nations High Commissioner for Refugees (UNHCR) and a local NGO Empowering Children Foundation (Fundacja Dajemy Dzieciom Siłę) to support the Child Protection Working Group. This collaboration aimed to strengthen systems and policies to protect Ukrainian children from violence, exploitation, and abuse.[54] Through coordinated efforts, the group provided guidance, training, and resources to frontline workers, while ensuring that child protection services were accessible and responsive. By combining international expertise, local knowledge, and government support, the initiative enhanced the safety and well-being of displaced children across Poland.

Polish organizations work closely with UNICEF to support the Basic Needs and Health Sectors, aiming to harmonize the country-

wide response for refugee infants and children. Their efforts focus on ensuring access to quality, inclusive education within the national school system, as well as through informal education initiatives. At the same time, they strengthen social protection mechanisms to address children's health, safety, and overall well-being. By coordinating services across education, health, and social sectors, these partnerships have helped create a comprehensive and consistent support system for Ukrainian refugee children.

Poland has worked closely with UNICEF to respond to the urgent needs of Ukrainian refugees and to scale up emergency responses. Together, they have expanded access to healthcare, education, child protection, and psychosocial support, while providing emergency supplies and resources to families in crisis. This collaboration also involves training frontline workers, strengthening national systems, and coordinating with local authorities, NGOs, and community networks. By scaling up these efforts, Poland and UNICEF have been able to deliver timely and effective assistance to a large number of displaced children and families across the country.

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In other efforts, the Polish government has also facilitated coordination between various government entities and the UNHCR, International Organization for Migration (IOM), World Health Organization (WHO), and International Rescue Committee (IRC). By creating clear communication channels, joint planning mechanisms, and shared operational frameworks, the government ensured that resources, information, and services were effectively coordinated. This collaboration minimized duplication, improved efficiency, and allowed humanitarian organizations to complement state-led initiatives, providing comprehensive support in areas such as protection, health, education, and social services for Ukrainian refugees.

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Guidance from Various Multinational Organizations

The European Union (EU)'s legal and regulatory framework, combined with Poland's country-specific practices, required a thorough understanding of the rules of engagement and the operational context for the refugee response. Humanitarian actors, government agencies, and NGOs needed to navigate EU asylum policies, temporary protection directives, and national regulations to provide effective support. This included understanding eligibility for services, legal status, and protection rights for refugees, as well as coordinating across multiple institutions and sectors. A clear grasp of both EU-wide and local procedures was essential to ensure social assistance, for healthcare, education, and social protection, was delivered efficiently and in compliance with legal requirements.

United Nations Children's Fund has been able to tap into the capacity of the Polish national and regional governments, combining it with its own technical expertise to strengthen the refugee response. By working alongside government institutions, it has helped design and implement programs in education, health, child protection, and social services. This approach has helped scale up emergency programs, such as access to schools, early childhood care, vaccinations, and psychosocial support, while leveraging the capacity and expertise of local authorities. Using the state's service delivery systems ensured that refugee assistance was integrated, sustainable, and aligned with national structures. This collaboration has also promoted efficient use of existing infrastructure, personnel, and administrative systems. The partnership has ensured that interventions were both locally feasible and aligned with international standards, maximizing the impact of assistance for Ukrainian children and families in Poland.

United Nations Children's Fund Poland have partnered with 12 municipalities that host around 75% of the total Ukrainian refugee population in the country.[56] Through this collaboration, UNICEF supported local authorities in providing education, healthcare, psychosocial services, and child protection programs. The partnership helped municipalities coordinate resources, train staff, and create safe spaces for children and families. By focusing on areas with the highest concentration of refugees, these initiatives maximized impact and ensured that displaced Ukrainians received timely and effective support.

United Nations Children's Fund Poland has also scaled up the delivery of humanitarian support to meet the urgent needs of Ukrainian refugees. This included strengthening the capacities of frontline responders, providing emergency supplies, and mobilizing

human resources to assist children and families. United Nations Children's Fund worked to support national systems, ensuring that healthcare, education, and social services could cope with increased demand. These efforts were carried out in close coordination with the Polish government, other UN agencies, the Polish National Committee (NatCom), as well as youth and community networks. By combining resources, expertise, and local knowledge, UNICEF helped deliver timely and effective assistance to those most affected by the crisis.[13]

Facilitated coordination between various units of the Polish government has helped streamline the efforts in refugee response with leading organizations such as UNHCR, IOM, WHO, and IRC. By creating clear communication channels, joint planning mechanisms, and shared operational frameworks, the government ensured that resources, information, and services were effectively coordinated. This collaboration minimized duplication, improved efficiency, and allowed humanitarian organizations to complement state-led initiatives, providing comprehensive support in areas such as protection, health, education, and social services.

Governmental Budgetary Prioritization for Mother-infant Care

Poland allocates a substantial portion of its national health budget to maternal and child health, including prenatal care, neonatal services, vaccinations, and specialized care for premature or high-risk infants, ensuring broad access and high-quality services. In contrast, Ukraine's healthcare budget for infants has historically been more limited, with fewer resources for prenatal screening, neonatal intensive care, and preventive programs, and the ongoing conflict has further strained funding and service delivery. These differences mean that while Polish infants generally benefit from well-supported healthcare systems, Ukrainian infants, particularly those in conflict-affected areas, face greater challenges in accessing timely and comprehensive medical care.

Epilogue

The arrival of Ukrainian infants and young families in Poland has highlighted both the resilience of displaced populations and the strength of coordinated humanitarian and healthcare responses. Despite the immense challenges posed by conflict, displacement, and disrupted prenatal and neonatal care, Polish authorities, healthcare providers, NGOs, and international organizations such as UNICEF have worked together to provide comprehensive support—ranging from medical care, vaccination, and neonatal interventions to psychosocial, educational, and social services.[57] Ukrainian infants, including those born prematurely or with congenital or infectious conditions, have benefited from timely interventions, specialized care, and protective services that have mitigated many of the risks associated with displacement. While ongoing efforts are needed to ensure continuity of care and long-term development, the experience underscores the importance of preparedness, collaboration, and culturally sensitive approaches in safeguarding the health and well-being of the youngest and most vulnerable refugees.

References

1. Domaradzki J, Walkowiak D, Bazan D, et al. Volunteering in the front line of the Ukrainian refugee crisis: A brief report from Poland. Front Public Health 2022;10:979751. DOI: 10.3389/fpubh.2022.979751.
2. Korzeniewski K, Shkilna M, Huk M, et al. Ukrainian war refugees and migrants in Poland: Implications for public health. J Travel Med 2024;31(1):taad119. DOI: 10.1093/jtm/taad119.
3. Nenko I, Baranowska B, Szlendak B, et al. "We were left to our own devices": Midwives' experiences of providing maternity care to Ukrainian women in Poland after the outbreak of the full-scale war in Ukraine. Women Birth 2024;37(4):101629. DOI: 10.1016/j.wombi.2024.101629.
4. UN High Commissioner for Refugees calls for immediate end to Ukraine war, which has uprooted over 10 million people | UNHCR [accessed 2026 Feb 24]. Available from: https://www.unhcr.org/news/press-releases/un-high-commissioner-refugees-calls-immediate-end-ukraine-war-which-has.
5. Situation Ukraine Refugee Situation [accessed 2026 Feb 24]. Available from: https://data.unhcr.org/en/situations/ukraine.
6. Ukrainian refugees by country 2025| Statista [accessed 2026 Feb 24]. Available from: https://www.statista.com/statistics/1312584/ukrainian-refugees-by-country/?srsltid=AfmBOopUpWfiVU-s42hpQNiUJhgTcl8Y6Ug8zxkqSbbuDtTo4SPKUE7I.
7. Kopeć K. Ukrainian refugees in Poland – number and distribution. J Geogr Politics Soc 2025;15(4):48–57. DOI: 10.26881/jpgs.2025.4.06.
8. Strzelecki P, Growiec J, Wyszyński R. How much are Ukrainian refugees contributing to the Polish economy? NBP Working Papers; 2025 [accessed 2026 Feb 24]. Available from: https://ideas.repec.org/p/nbp/nbpmis/376.html.
9. Stop attacks on health care in Ukraine [accessed 2026 Feb 24]. Available from: https://www.who.int/news/item/13-03-2022-stop-attacks-on-health-care-in-ukraine.
10. Conflict upends life in Ukraine | United Nations Population Fund [accessed 2026 Feb 24]. Available from: https://www.unfpa.org/conflict-upends-life-ukraine.
11. Statistics Poland/Topics/Statistical yearbooks/Statistical Yearbooks/Demographic Yearbook of Poland 2024 [accessed 2026 Feb 24]. Available from: https://stat.gov.pl/en/topics/statistical-yearbooks/statistical-yearbooks/demographic-yearbook-of-poland-2024,3,18.html.
12. UNFPA EECA | "We are here to take care of them": Midwives in Poland deliver comfort, care and community to Ukrainian refugee women [accessed 2026 Feb 24]. Available from: https://eeca.unfpa.org/en/news/%E2%80%9Cwe-are-here-take-care-them%E2%80%9D-midwives-poland-deliver-comfort-care-and-community-ukrainian?utm_source=chatgpt.com.
13. UNICEF Refugee Response Office in Poland | UNICEF Europe and Central Asia [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/poland?utm_source=chatgpt.com.
14. Perelli-Harris B, Gerber T, Hilevych Y. Uncertainty and fertility in Ukraine on the eve of Russia's full-scale invasion: The impact of armed conflict and economic crisis. Eur J Popul 2024;40(1):28. DOI: 10.1007/s10680-024-09713-7.
15. SDG – Report 2024 [accessed 2026 Feb 24]. Available from: https://raportsdg.stat.gov.pl/2024/en/Early_childhood.html?utm_source=chatgpt.com.
16. Ukraine Infant Mortality Rate (1950–2025) [accessed 2026 Feb 24]. Available from: https://www.macrotrends.net/global-metrics/countries/ukr/ukraine/infant-mortality-rate.
17. Birth rates in Poland vs Ukraine: 1960–2023 data [accessed 2026 Feb 24]. Available from: https://georank.org/birth-rate/poland/ukraine?utm_source=chatgpt.com.
18. Oleksyk TK, Wolfsberger WW, Weber AM, et al. Genome diversity in Ukraine. Gigascience 2021;10(1):giaa159. DOI: 10.1093/gigascience/giaa159.
19. Neonatal screening revealed 646 diseases in infants: Ministry of Health reminds of the importance of diagnostics in Ukraine, neonatal screening, which allows for the early detection of serious congenital and hereditary diseases in newborns, has identifie… [accessed 2026 Feb 24]. Available from: https://unn.ua/en/news/neonatal-screening-

revealed-646-diseases-in-infants-the-ministry-of-health-reminded-of-the-importance-of-diagnostics?utm_source=chatgpt.com.
20. Keasley J, Blickwedel J, Quenby S. Adverse effects of exposure to armed conflict on pregnancy: A systematic review. BMJ Glob Health 2017;2(4):e000377. DOI: 10.1136/bmjgh-2017-000377.
21. Programme Guidance on Maternal Nutrition | UNICEF [accessed 2026 Feb 24]. Available from: https://www.unicef.org/documents/programme-guidance-maternal-nutrition?utm_source=chatgpt.com.
22. Black RE, Victora CG, Walker SP, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. Lancet 2013;382(9890):427–451. DOI: 10.1016/S0140-6736(13)60937-X.
23. PL Poland – Social Support – United for Ukraine [accessed 2026 Feb 24]. Available from: https://www.ufuplatform.com/en-us/articles/5678533705751?utm_source=chatgpt.com.
24. Dale E, Bredenkamp C, Doroshenko O, et al. Health financing reform in Ukraine: Progress and future directions; 2022. Washington, DC: World Bank.
25. Regional Office for Europe W. WHO Country Office in Poland annual report 2024; 2025 [accessed 2026 Feb 24]. Available from: http://apps.who.int/bookorders.
26. Executive summary population: Ukrainian refugees to neighbouring refugee-hosting countries overview of crisis.
27. Prusaczyk A, Bogdan M, Vinker S, et al. Health care organization in Poland in light of the refugee crisis related to the military conflict in Ukraine. Int J Environ Res Public Health 2023;20(5):3831. DOI: 10.3390/ijerph20053831.
28. The number of abortions has decreased by 18% since the beginning of the major conflict — Opendatabot [accessed 2026 Feb 24]. Available from: https://opendatabot.ua/en/analytics/abortions-2023?utm_source=chatgpt.com.
29. How many Ukrainian women had an abortion during the Great War: The numbers that statistics hide – Komersant Ukrainian [accessed 2026 Feb 24]. Available from: https://komersant.ua/en/skilky-ukrainok-zrobyly-abort-pid-chas-velykoi-viyny-tsyfry-iaki-prykhovuie-statystyka/?utm_source=chatgpt.com.
30. Ukrainians in Poland – statistics & facts | Statista [accessed 2026 Feb 24]. Available from: https://www.statista.com/topics/10586/ukrainians-in-poland/?utm_source=chatgpt.com.
31. Rodríguez-Muñoz MF, Chrzan-Dętkoś M. Refugee women and perinatal mental health: The experience of war. Arch Womens Ment Health 2025;28(2):197–199. DOI: 10.1007/s00737-025-01576-z.
32. Medevac Ukraine [accessed 2026 Feb 24]. Available from: https://www.medevac-ukraine.com/en/?utm_source=chatgpt.com.
33. Over 5000 patients transported through Ukraine's medevac programme: Baby Sofiia's story [accessed 2026 Feb 24]. Available from: https://www.who.int/europe/news-room/feature-stories/item/over-5000-patients-transported-through-ukraine-s-medevac-programme--baby-sofiia-s-story?utm_source=chatgpt.com.
34. Behboudi-Gandevani S, Bidhendi-Yarandi R, Panahi MH, et al. Perinatal and neonatal outcomes in immigrants from conflict-zone countries: A systematic review and meta-analysis of observational studies. Front Public Health 2022;10:766943. DOI: 10.3389/fpubh.2022.766943.
35. Rodríguez-Muñoz MF, Chrzan-Dętkoś M, Uka A, et al. The impact of the war in Ukraine on the perinatal period: Perinatal mental health for refugee women (pmh-rw) protocol. Front Psychol 2023;14:1152478. DOI: 10.3389/fpsyg.2023.1152478.
36. Janczewska I, Jassem-Bobowicz J, Hinca K, et al. Group B streptococcus colonization status and antibiotic use during labour – A single-centre observational study. Ginekol Pol 2024;95(7):549–556. DOI: 10.5603/gpl.95616.
37. Trijbels-Smeulders MAJM, Kollée LAA, Adriaanse AH, et al. Neonatal group B streptococcal infection: Incidence and strategies for prevention in Europe. Pediatr Infect Dis J 2004;23(2 Suppl):172–173. DOI: 10.1097/01.inf.0000111212.94913.5f.
38. Liu Y, Ai H. Current research update on group B streptococcal infection related to obstetrics and gynecology. Front Pharmacol. 2024;15:1395673. DOI: 10.3389/fphar.2024.1395673.
39. Manuel G, Twentyman J, Noble K, et al. Group B streptococcal infections in pregnancy and early life. Clin Microbiol Rev 2024;38(1):e0015422. DOI: 10.1128/cmr.00154-22.
40. Jaan A, Rajnik M. TORCH complex. StatPearls; 2023 [accessed 2026 Feb 24]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK560528/.
41. Intrauterine (Congenital) Infections [accessed 2026 Feb 24]. Available from: https://www.utmb.edu/Pedi_Ed/CoreV2/Neonatology/Neonatology31.html?utm_source=chatgpt.com.
42. Lewtak K, Nitsch-Osuch A, Dzielska A, et al. Healthcare professionals' perspectives on the challenges in the vaccination of Ukrainian child migrants and war refugees: Findings from a qualitative study in Poland. Int J Occup Med Environ Health 2024;37(6):602. DOI: 10.13075/ijomeh.1896.02478.
43. Lewtak K, Mazur J, Dwyer H, et al. Determinants of Ukrainian mothers' intentions to vaccinate their children in Poland: A cross-sectional study. Vaccines (Basel) 2025;13(3):325. DOI: 10.3390/vaccines13030325.
44. UNICEF, the Ministry of National Education and humanitarian partners launch "Back to Learning" campaign to support enrolment and integration of refugee children in Polish schools [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/press-releases/unicef-ministry-national-education-and-humanitarian-partners-launch-back-learning?utm_source=chatgpt.com.
45. UNICEF and Ministry of National Education sign 2025 work plan to support the education of refugee children from Ukraine [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/press-releases/unicef-and-ministry-national-education-sign-2025-work-plan?utm_source=chatgpt.com.
46. Educational integration of refugee children in Polish schools. Where do we stand after the introduction of compulsory schooling? [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/press-releases/educational-integration-refugee-children?utm_source=chatgpt.com.
47. Temporary care of a child from Ukraine in Poland | Pravovsim.org | Pravovsim.org [accessed 2026 Feb 24]. Available from: https://pravovsim.org/en/inform-portal/post/temporary-care-of-a-child-from-ukraine-in-poland?utm_source=chatgpt.com.
48. With $4 Billion in Tax Contributions, Ukrainians in Poland Give Far More Than They Receive [accessed 2026 Feb 24]. Available from: https://www.forbes.com/sites/lidiakurasinska/2025/03/14/with-4-billion-in-tax-contributions-ukrainians-in-poland-give-far-more-than-they-receive/?utm_source=chatgpt.com.
49. How Ukrainians are boosting Poland's economy and solving its problems | European Pravda [accessed 2026 Feb 24]. Available from: https://www.eurointegration.com.ua/eng/news/2025/04/23/7210055/.
50. HOPE AMID CRISIS one year of UNICEF's response in Poland for children and families fleeing war in Ukraine; 2022 [accessed 2026 Feb 24]. Available from: www.unicef.org/eca/poland.
51. Healing away from home | UNICEF Europe and Central Asia [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/stories/healing-away-home?utm_source=chatgpt.com.
52. UNICEF and the Polish Ministry of Education and Science promote inclusive education [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/press-releases/unicef-and-polish-ministry-education-and-science-promote-inclusive-education?utm_source=chatgpt.com.
53. Ministry of Family, Labour and Social Policy and UNICEF strengthen ongoing partnership for refugee children in Poland [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/press-releases/ministry-family-and-unicef-strengthen-partnership?utm_source=chatgpt.com.

54. The Refugee Agency UU. Poland bi-annual fact sheet: September 2024; 2024 [accessed 2026 Feb 24]. Available from: www.unhcr.org|.
55. Poland | Save the Children International [accessed 2026 Feb 24]. Available from: https://www.savethechildren.net/poland.
56. Innovative partnerships with municipalities in Europe ensure that no refugee child is left behind | UNICEF Europe and Central Asia [accessed 2026 Feb 24]. Available from: https://www.unicef.org/eca/stories/innovative-partnerships-municipalities-europe-ensure-no-refugee-child-left-behind?utm_source=chatgpt.com.
57. Mogilevkina I, Dobryanskyy D, MacDonald R, et al. A descriptive analysis of a medical humanitarian aid initiative for quality perinatal management in war-torn Ukraine. Confl Health 2025;19(1):6. DOI: 10.1186/s13031-025-00644-6.

REVIEW ARTICLE

Calprotectin: An Overview

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Received on: 23 January 2026; *Accepted on*: 24 February 2026; *Published on*: 08 April 2026

Abstract

Calprotectin is a heterodimeric calcium- and zinc-binding protein comprised of two calcium-binding peptides, *S100A8* and *S100A9*. It is expressed mainly in activated neutrophils and macrophages and serves as a sensitive biomarker of inflammation. It promotes innate immunity by limiting microbial growth through depletion of essential metals such as zinc and manganese, and by activating signaling pathways involving the toll-like receptor 4 (TLR4) and the receptor for advanced glycation end (RAGE) products. Neonates depend to a great extent on innate immunity for survival and can show higher calprotectin levels than older children and adults. In addition to the gastrointestinal tract, calprotectin is detectable in all anatomical compartments, whenever and wherever neutrophils or activated macrophages are recruited. High circulating levels of calprotectin are detectable during systemic inflammatory response syndrome, further highlighting its important contribution to immune responses. In patients with gut disorders, fecal calprotectin is a useful noninvasive marker that can help differentiate inflammatory bowel conditions from functional gastrointestinal disorders, assess the severity of disease activity, and monitor therapeutic responses. Calprotectin has been viewed as a highly conserved component of the innate immune system for over 500 million years. Clinically, it is a valuable diagnostic and monitoring tool that enhances decision-making while reducing reliance on invasive investigations. In this article, we present our current understanding of calprotectin expression, evolution, and clinical importance. We have added data from our own preclinical studies to an extensive review of the literature using the databases PubMed, EMBASE, and Scopus.

Keywords: Adenine complex ATP-binding cassette, Alarmins, ATP-binding cassette, *Calgranulin-A*, *Calgranulin B*, Citrullination, Cytosolic protein, CzcD, EF-hand, Electrostatic interactions, Filaggrin, Heterodimer geometry, High mobility group box 1, Hydrophobic interactions, Inflammatory stimuli, Manganese transport protein H, Myeloid-related protein-14, Myeloid-related protein-8, Neonatal intensive care unit, Neonate, Neutrophil extracellular trap, Paralogous proximity, Periplasmic binding protein, Pneumococcal surface antigen B, C, and A, Pseudopaline, Receptor for advanced glycation end products, *S100A8*, *S100A9*, *Salmonella* iron transporter ABCD, Siderophores, Transition metal sequestration activity, Zinc uptake regulator, Zincophores, Zinc uptake ATP-binding box complex.

Newborn (2026): 10.5005/jp-journals-11002-0153

Keypoints

- Calprotectin is a multifunctional cytosolic protein complex expressed in neutrophils, monocytes, macrophages, dendritic cells, keratinocytes, and squamous mucosal epithelial cells and is upregulated in response to inflammatory stimuli.
- Calprotectin is a heterodimeric protein complex comprised of two calcium-binding protein components, *S100A8* and *S100A9*. It promotes innate immunity by limiting microbial growth by depleting essential metals such as zinc and manganese, and inducing inflammatory responses by activating the toll-like receptor 4 (TLR4) and the receptor for advanced glycation end (RAGE) products.
- The antimicrobial activity of calprotectin has at least three mechanisms: (1) metal sequestration; (2) electrostatic interactions between its cationic surface regions and the anionic phospholipids in microbial membranes; and (3) hydrophobic interactions with microbial membranes.
- Calprotectin is a highly conserved component of the innate immune system originating from an ancestral protein in jawless fish about 500 million years ago.
- Neonates typically have higher serum (0.79 ± 0.33 µg/mL) and fecal calprotectin (0.34 ± 0.68 mg/gm stool) concentrations than those seen in adults. Levels gradually decrease with age and stabilize as the immune system undergoes maturation. Infants with sepsis, necrotizing enterocolitis (NEC), hypoxic stress, or infection show higher calprotectin levels in blood and stool.

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Introduction

Calprotectin is a multifunctional cytosolic protein complex expressed variably across different cell types.[1–4] It is expressed in neutrophils, monocytes, macrophages, dendritic cells, keratinocytes, and squamous mucosal epithelial cells, and is upregulated in response to inflammatory stimuli.[5] There are two calcium-binding protein components: (1) soluble in 100% saturated ammonium sulfate solution-A8 (*S100A8*) [named variously as *calgranulin-A*, migration inhibitory factor-related protein-8, or myeloid-related protein-8 (MRP8)] and (2) *S100A9* (*calgranulin B* or MRP14).[6–10] The two genes are highly conserved across all vertebrates, reflecting strong underlying evolutionary pressures to maintain metal-binding and antimicrobial functions; both are crucial for maintaining the innate immune and intestinal inflammatory responses.[11,12] *S100A8* and *S100A9* encode proteins with calcium-binding motifs and residues critical for transition metal sequestration, which together enable calprotectin to form functional heterodimers and heterotetramers.[13–16] And genetic variation, such as single-nucleotide polymorphisms, can influence calprotectin levels and activity.[17–19]

Calprotectin promotes innate immunity by (1) limiting microbial growth by depleting essential metals such as zinc (Zn) and manganese (Mn) and (2) inducing inflammatory responses by activating the TLR4 and the RAGE products.[20–24] It also works synergistically with various alarmins that carry damage-associated molecular patterns (DAMPs), such as the high mobility group box 1 (HMGB1; Fig. 1).[25–28] Not surprisingly, as an antimicrobial peptide, calprotectin inhibits the growth of many gram-positive and gram-negative bacteria, and fungi such as *Candida* spp.[29] Neonates, who depend on innate immunity for survival, often show higher calprotectin levels than older children and adults; the median calprotectin levels in neonatal stool can be 200–2,000 µg/gm and can rise further with mucosal inflammation.[30] In this article, we present our current understanding of calprotectin expression, evolution, and clinical importance. We have added data from our own preclinical and peer-reviewed studies to an extensive review of the literature using the databases PubMed, EMBASE, and Scopus.[31,32]

Structure of Calprotectin

Calprotectin is a heterodimeric protein complex comprised of an *S100A8* (MRP8) and another *S100A9* (MRP14) subunit.[33,34] Both protein motifs have been visualized with a thumb (end digit, E) and a forefinger (F) joined in a distal palm-like shape, the "EF-hand."[13,15] This distal palm is a short loop of about 12 amino acids and holds a "marble," a Ca^{++} ion (Fig. 2).[35] The presence of calcium ions induces conformational changes in the protein complex and exposes specific domains that can sense intracellular calcium fluctuations:[35,36]

- *N*-terminal EF-hand: A "pseudo" or *S100*-specific loop typically consisting of 14 amino acids, with a relatively limited Ca^{++} affinity.[37]
- *C*-terminal EF-hand: A "canonical" or classical EF-hand loop consisting of 12 amino acids, which exhibits higher calcium affinity.[38]

The properties of the calprotectin protein are detailed in Figure 3.[39–42] The protein is relatively water-soluble, and the hydrophobic amino acids are buried inside the core, away from the surface (Figs 3A and B).[24,43] The secondary structure (Fig. 3C), the three-dimensional distribution of various amino acids (Fig. 3D), and the relative proximity to Ca^{++} ions are reasonably well-characterized (Fig. 3E).[24,44] In the presence of Ca^{++} ions, calprotectin can form heterotetramers or higher-order oligomers, which can further enhance its stability and metal-binding capacity (Fig. 4).[45] The binding sites with Ca^{++} and other transitional metals such as Zn^{++} (Fig. 5) and Mn^{++} ions are known.[24,46,47] We know that the combination of Ca^{++}-induced conformational flexibility and metal sequestration underlies its antimicrobial effects, as these expose hydrophobic surfaces, allowing the protein to interact with various enzymes and ion channels.[48,49]

S100A8 and *S100A9* are expressed in neutrophils, monocytes, and activated macrophages, where these induce cytokine expression, activate leukocytes, and enhance antimicrobial activity as mediators of innate immunity and inflammation.[45,50–52] In addition to the gastrointestinal tract, these can be identified in the lungs, liver, skin, joints, and cardiovascular system, whenever and wherever neutrophils or activated macrophages are recruited.[53] These contribute to local antimicrobial defense, metal sequestration, and pro-inflammatory signaling.[9] Circulating calprotectin is detectable during systemic inflammatory response syndrome, further highlighting its important role in immune responses and inflammatory diseases.[21]

Tertiary/Quaternary Protein Structure

- Transition metal-binding sites: Mutations in the sequence for histidine, aspartate, or cysteine residues involved in sequestration of Zn and Mn can compromise nutritional immunity, diminishing antimicrobial activity against bacteria and fungi.[54]

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How to cite this article: Maheshwari A, Mohammadabadi T, Michie C. Calprotectin: An Overview. Newborn 2026;5(1):39–57.

Source of support: Nil

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

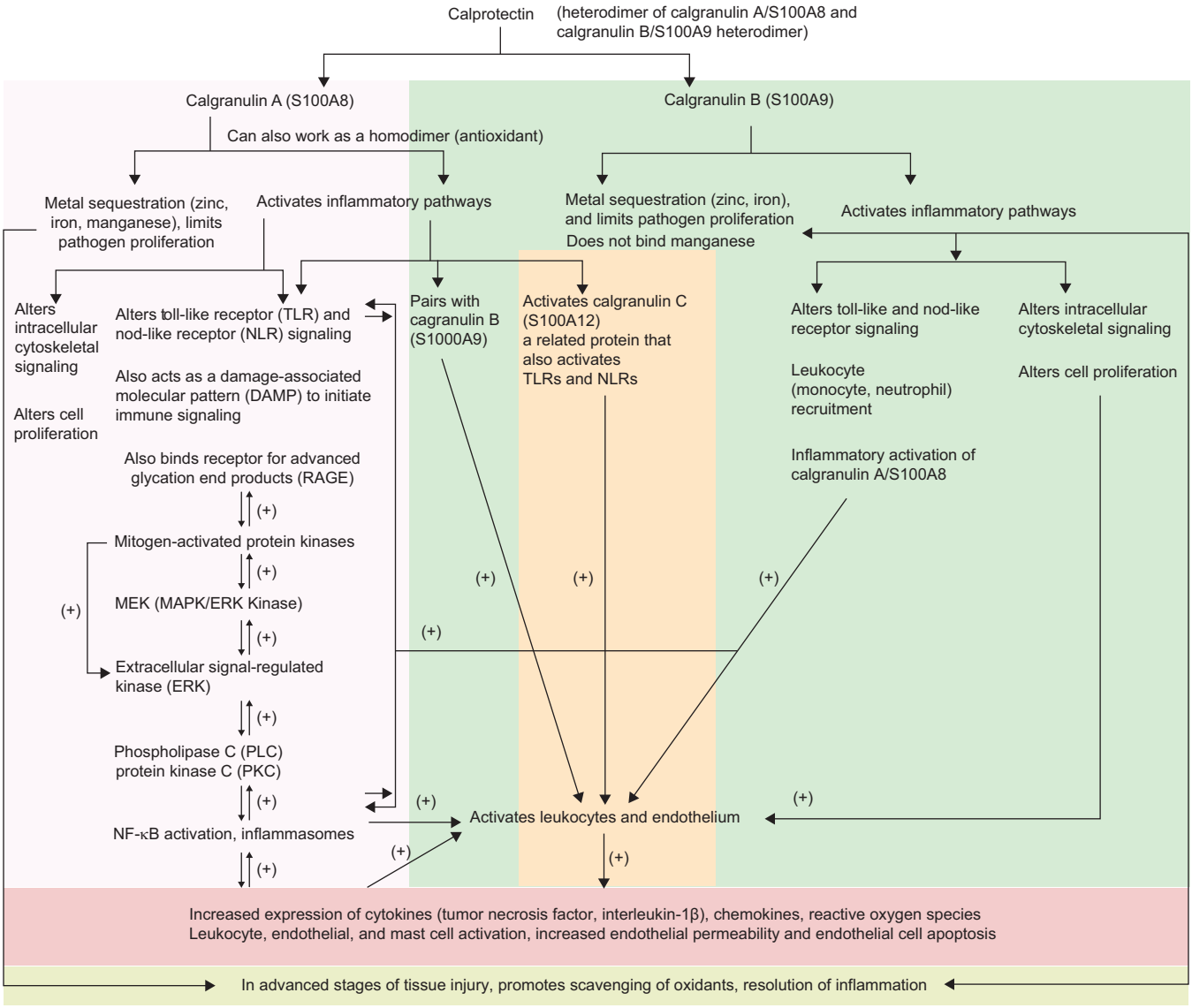
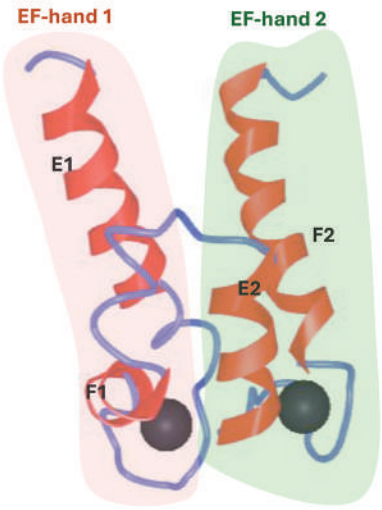


Fig. 1: Calprotectin is an important mediator in innate immunity; it is expressed predominantly in neutrophils and monocytes, where it contributes to early host defense against infection. Calprotectin is composed of *calgranulin A* (*S100A8*) and *calgranulin B* (*S100A9*) proteins. This complex promotes calcium-related signaling and immune function. Ca^{2+} binding induces conformational changes such as dimerization, increases its stability, its interaction with target molecules, and association with cell membranes during inflammation. In this calcium-bound state, calprotectin sequesters transition metals such as zinc and manganese more effectively, strengthening its antimicrobial activity and role in innate immune defense. Calprotectin is released at sites of inflammation and exerts antimicrobial activity by sequestering essential metal ions such as zinc and manganese and thereby limiting microbial growth (nutritional immunity). In addition to its antimicrobial role, calprotectin also functions as a DAMP, activating pattern recognition receptors and amplifying inflammatory signaling. Through these mechanisms, calprotectin helps coordinate innate immune responses and serves as a sensitive biomarker of inflammation

- Protein–protein interaction surfaces: Changes at the *S100A8/S100A9* interface in calprotectin may alter the tertiary/quaternary structure.[45] Appropriate heterodimerization is critical for the stability and function of calprotectin. The heterodimers develop with specific hydrophobic and electrostatic interactions, and are needed for the stability of the protein complex, sequestration of metal ions, and interaction with microbial membranes.[5,20,55] These effects are particularly relevant in neonates and during development, where subtle differences at the interface may modulate calprotectin activity and influence early gut colonization and immune defense.[56]
- Interaction with metallic ions: Heterodimerization positions the histidine- and aspartate-coordinated metal-binding sites at the subunit interface for chelation of Zn, Mn, and other transition metals.[44] Alterations at the interface due to mutations, post-translational modifications, or structural instability can distort the heterodimer and its metal-binding sites.[20,57] This effect is important in the neonates, where it might influence host–microbe interactions.
- Interactions with microbial membranes: The *S100A8/S100A9* interface is critical for interactions with microbial membranes.[46] Proper heterodimerization positions key hydrophobic and



Calgranulin A (S100A8)

KEY STRUCTURAL ELEMENTS

S100A8

Cytosolic protein, no signal peptides; Forms a heterodimer with S100A9 to perform metal sequestration, antimicrobial activity, and pro-inflammatory signaling via receptors like RAGE and TLR4.

Secreted via the endoplasmic reticulum–Golgi pathway. Non-classical release from leukocytes via non-classical secretion such as passive release during cell activation/necrosis or via microvesicles/exosomes.

Two EF-hand domains:

- N-terminal EF-hand (pseudo-EF-hand): 14-residue motif, low-affinity calcium binding, overall structural stability. Asp10, Asp12, and Glu14 coordinate calcium with lower affinity.
- C-terminal EF-hand (canonical-EF-hand): 12-residue motif, higher-affinity calcium binding with conformational change. Asp57, Asp59, Glu62, and Glu66 coordinate calcium with high affinity, driving conformational changes.

Calcium-binding residues: aspartate (Asp), glutamate (Glu), and sometimes asparagine (Asn) residues, which coordinate the calcium ion in a pentagonal bipyramidal geometry. Calcium binding leads to an 'open' conformational change to expose a hydrophobic surface and heterodimerization with S100A9 and enabling interactions with target proteins. "Closed" S100A8 is largely inactive.

EF-hand loops contain conserved acidic residues, aspartate and glutamate, which coordinate the calcium ion in a pentagonal bipyramidal geometry. Calcium binding in S100A8 induces a structural rearrangement in S100A9, exposing hydrophobic surfaces that allow heterodimerization to form calprotectin and facilitate interactions with receptors such as RAGE and TLR4. Without calcium, S100A9 remains largely inactive. These cation-binding sites are essential for its immune and inflammatory functions.

Zinc-binding sites needed for antimicrobial activity. Zinc coordination occurs at interface regions when S100A8 forms a heterodimer with S100A9. Zinc-binding residues include conserved histidine (His) and aspartate (Asp) residues; in S100A8, residues such as His17, His27, and Asp30 participate in metal ion coordination at the heterodimer interface with S100A9, enabling nutritional immunity.

There is a heterodimer requirement; zinc binding is more stable when S100A8 pairs with S100A9 to form calprotectin, creating a tetrahedral coordination site at the dimer interface. This metal-chelating activity inhibits microbial growth through nutritional immunity.

Antimicrobial activity: Zinc-binding sites contribute to antimicrobial activity through metal ion sequestration and structural interactions within the calprotectin heterodimer.

- N-terminal EF-hand has lower calcium affinity but stabilizes the overall fold of the protein. Antimicrobial activity is mediated at the heterodimer interface, where conserved histidine and aspartate residues from S100A8 coordinate zinc and manganese ions. These changes deprive pathogens of essential metals and provide nutritional immunity.
- C-terminal EF-hand domain in S100A8 binds calcium and exposes hydrophobic surfaces needed for heterodimerization with S100A9. These metal-binding regions, combined with the conformational flexibility conferred by the EF-hand domains, define the functional domains of S100A8 that are critical for its direct antimicrobial effects and innate immune defense.

Dimerization and structural stabilization: Residues within the hydrophobic surface exposed upon calcium binding, including portions of the C-terminal EF-hand, are critical for heterodimer formation with S100A9 and proper structural integrity.

Functional motifs: Conserved motifs in both EF-hands contribute to receptor interaction (such as RAGE, TLR4) and pro-inflammatory signaling.

Active site: the term is used in the context of metal-binding and functional activity, rather than enzymatic catalysis, because S100A8 is not an enzyme:

- ***Two EF-hand domains:*** *N*-terminal pseudo-EF-hand (low-affinity) that contributes to structural stability; and C-terminal canonical EF-hand (high-affinity) that undergoes conformational changes upon calcium binding, exposing hydrophobic surfaces that enable heterodimerization with S100A9.
- ***Transition metal-binding site (zinc/manganese):*** located at the heterodimer interface with S100A9. Key residues include His17, His27, and Asp30, which coordinate zinc or manganese ions; site crucial for **nutritional immunity**, as it sequesters essential metals from pathogens.
- ***Functional interface for immune signaling:*** calcium-induced structural changes expose surfaces that allow interaction with receptors such as RAGE and TLR4, mediating pro-inflammatory signaling.

Sequence splicing: process for removal of introns; exons are joined in the pre-mRNA transcript and the mature mRNA is then translated.

S100A8 gene shows 3 exons and 2 introns. Canonical splicing produces the full-length protein, including both EF-hand calcium-binding domains necessary for structural stability, heterodimerization with S100A9, and antimicrobial activity.

Alternative splicing appears to be rare or minimal. Proper splicing is crucial because any mis-splicing or exon skipping could disrupt the EF-hand domains or the metal-binding interface, impairing calcium and zinc binding, heterodimer formation, and overall innate immune function.

Post-translational modifications: several amino acid residues undergo such changes and affect its structure, activity, and interactions. Key modified residue positions include:

- ***Cysteine residues:*** Cys42 is prone to oxidation, forming disulfide bonds or sulfenic/sulfinic acids under oxidative stress. This modification can influence protein stability and pro-inflammatory activity.
- ***Serine/threonine residues:*** Ser100 (numbering may vary by species) can undergo phosphorylation, which modulates calcium-dependent conformational changes and interaction with S100A9 or other target proteins.
- ***Methionine residues:*** Certain methionines may undergo oxidation, which can alter the protein's redox state and antimicrobial function.
- ***Other modifications:*** Acetylation and nitrosylation at exposed lysine or cysteine residues have been reported in inflammatory contexts, affecting immune signaling and receptor interactions.

Genetic alterations:
Two non-coding or regulatory mutations, rs3795391 (A > G) and rs3806232 (A > G) located upstream of the encoding gene, have been associated with increased plasma levels of S100A8 and systemic inflammation.*

Specific minor allele frequencies (MAFs) vary by population, though precise numeric frequencies are not known. These SNPs are common enough to be counted in association studies across ethnic groups.

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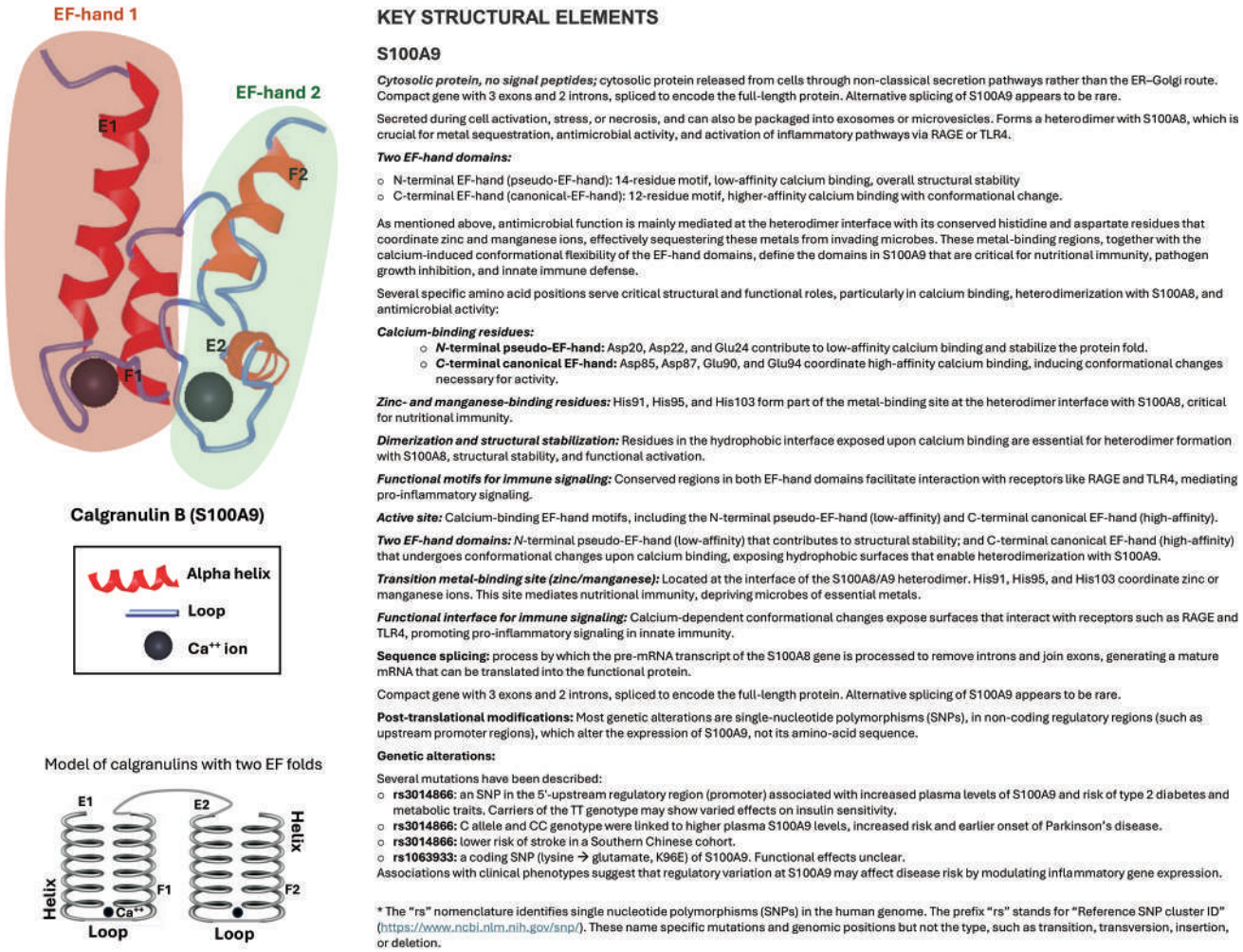


Fig. 2: *Calgranulin A* and *B* are both comprised of two helix-loop-helix EF hands. The two helices are visualized as digits: a thumb (end digit, E) and a forefinger (F). These digits are joined on an imagined palm, a short loop of about 12 amino acids, which holds a Ca^{++} ion. A low-affinity calcium-binding site is also seen at the *N*-termini on the helices and a high-affinity site at the C-termini. Calcium binding induces a conformational change with helical rotation, exposing a hydrophobic surface for interaction with target proteins such as RAGE products

cationic regions that interact with negatively charged bacterial membranes.[46] Altered conformation can reduce the binding with the microbial membranes and its antimicrobial activity.[29]

Functional defects in calprotectin could theoretically increase susceptibility to infections or inflammation, though naturally occurring human mutations are not widely reported.[58] One reason for the genetic mutations not being widely reported in a clinical context is that calprotectin is usually marked as a biomarker for inflammation and measured for concentrations, not function.[6]

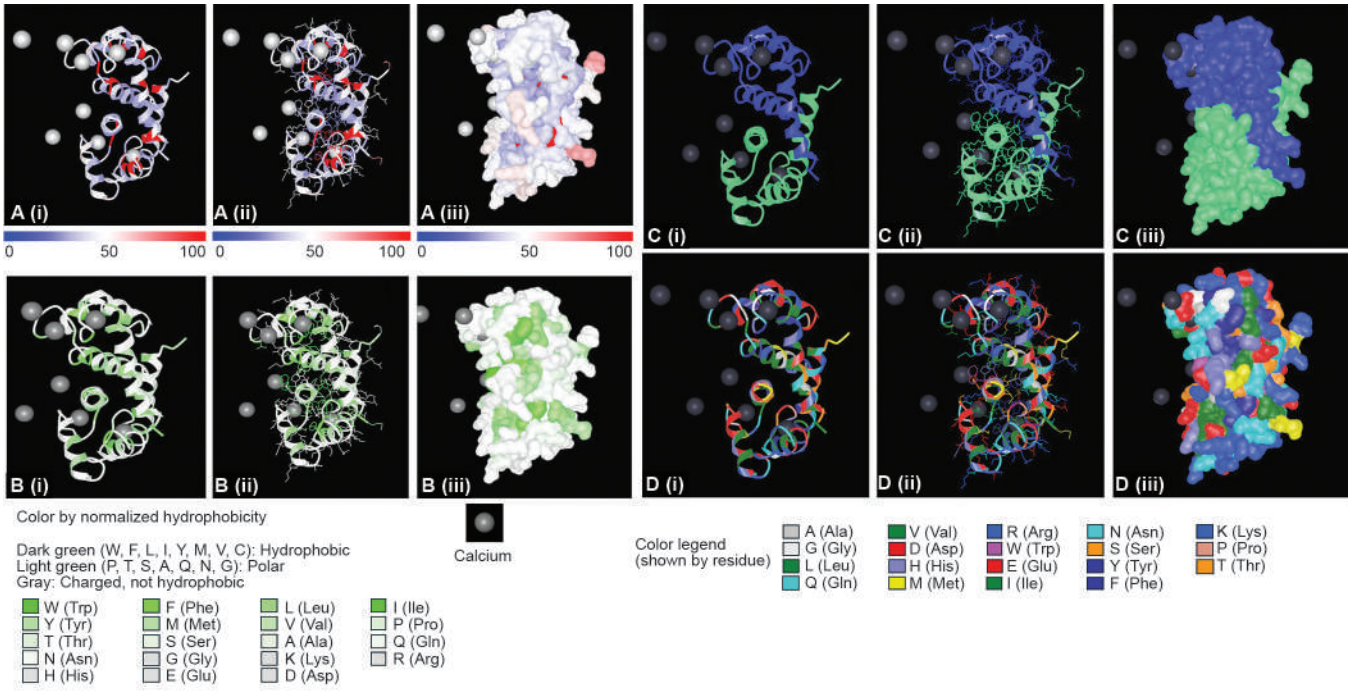
Metal-binding Domains

Ca^{++} ions bind EF-hand motifs in calprotectin and induce conformational changes that stabilize it and promote dimer/tetramer formation, indirectly enhancing its transition metal sequestration activity.[24] There are two metal-binding sites at the interface of the *S100A8/S100A9* heterodimer, involving histidine (His) and aspartate (Asp) residues from both subunits:[14] (1) a high-affinity multi-His motif binds divalent metal ions such as Zn^{++}; Fig. 5B), Mn^{++}, ferrous (Fe^{++}), and nickel (Ni^{++});[14] and (2) a combination of His and Asp residues sequesters Zn^{++}.[20] These metals stabilize the heterodimer geometry and enhance the affinity and specificity of metal sequestration in a feed-forward process.[20,24]

Metal ions are central to the structure and function of calprotectin.[59] As mentioned above, Ca^{++}-enhanced inhibition of microbial enzymes and cellular survival.[47] Metal binding also promotes protein oligomerization and may facilitate membrane destabilization, further enhancing antimicrobial effects.[60] Thus, metal ions are both structural cofactors and functional effectors, enabling calprotectin to regulate microbial growth and contribute to host defense.[20]

Mechanisms of Secretion

Calprotectin is secreted primarily by activated neutrophils and monocytes/inflammatory macrophages.[61] It lacks a signal peptide, and


A (i)
A (ii)
A (iii)
0 50 100
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B (i)
B (ii)
B (iii)
C (i)
C (ii)
C (iii)
D (i)
D (ii)
D (iii)
Color by normalized hydrophobicity
Dark green (W, F, L, I, Y, M, V, C): Hydrophobic
Light green (P, T, S, A, Q, N, G): Polar
Gray: Charged, not hydrophobic
W (Trp)
Y (Tyr)
T (Thr)
N (Asn)
H (His)
F (Phe)
M (Met)
S (Ser)
G (Gly)
E (Glu)
L (Leu)
V (Val)
A (Ala)
K (Lys)
D (Asp)
I (Ile)
P (Pro)
Q (Gln)
R (Arg)
Calcium
Color legend
(shown by residue)
A (Ala)
G (Gly)
L (Leu)
Q (Gln)
V (Val)
D (Asp)
H (His)
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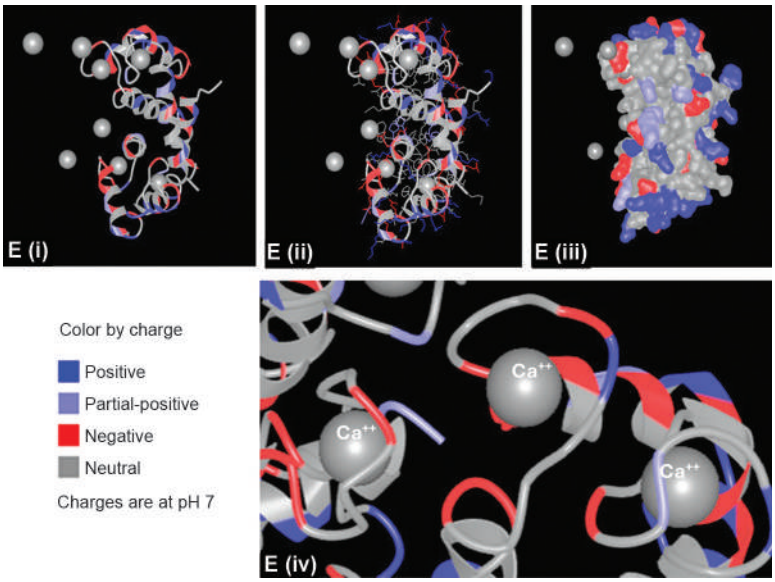


Figs 3A to E: This figure shows three sequential illustrations of the calprotectin molecule from left-to-right: (i) primary ribbon structures with folds show the organization of the protein backbone; (ii) structure with side chains; and (iii) molecular surface. (A) Solvent accessibility. Color change from blue-to-white-to-red indicates increasing solvent accessibility. The average accessible surface of residues in most folded proteins is around relative to an unfolded state. A value of suggests that the protein's surface is well-hydrated; (B) Hydrophobicity. Predicted solvent-accessible surface area (SASA), shown in a scale ranging from a deep green color at its low end to high white: (i–ii) primary ribbon structure and the structure with side-chains shows the green and white color residues as evenly distributed; (iii) solvent-accessible space-filling model predicts these surfaces as much whiter and hence, the hydrophobic residues green residues are buried away from the surface inside the core of the protein. We have used the SASA method to produce this illustration. Relative solvent accessible area (RSA)/SASA are standard measures to describe the degree of residue exposure in the protein surface. There are many methods for the estimation of RSA/SASA that focus on the Ca atom-distance matrix and use deep learning methods; some of the existing RSA estimators are based on coordination numbers, half-sphere exposure, and SphereCon, one of the recognized RSA-estimation algorithms; (C) Calprotectin is a heterodimer of *calgranulin A* (*S100A8*, blue) and *calgranulin B* (*S100A9*, green); (D) Amino acid composition of the calprotectin heterodimer molecule. The amino acid color-coding is as shown below. (E) The amino acid structure (i–ii) showed a good overall balance of positively and negatively charged amino acid residues. However, (iii) the surface of the protein molecules showed more neutral/positively charged regions. Inside the molecule, (iv) most calcium ions (Ca^{++}) were located close to negatively charged glutamic acid, aspartic acid, asparagine, and glycine residues. Zinc (Zn^{++}) is seen at specific interfacial sites, coordinated by Histidine (H) and Cysteine (C) residues such as the His-X-X-X-His motifs on both *S100A8* and SI100A9 subunits (not shown here). The presence of Ca^{++} may enhance Zn-binding, which is crucial for the antimicrobial functions. In the EF-hand motif, the glutamic and aspartic acids in the loop region provide the negatively charged oxygen atoms, which enhance the effect of the positively charged Ca^{2+} ions. Here, Ca^{++} may induce a significant conformational and charge alteration to promote sequestration of transition metals and self-association into a tetramer. Each calprotectin subunit (*S100A8* and *S100A9*) has two EF-hands; the *C*-terminal EF-hand is considered "canonical" and has a higher negative charge and a stronger affinity for Ca^{2+} ions. The *N*-terminal EF -hand is "non-canonical," with a lower number of negatively charged residues and binds Ca^{++} with lower affinity

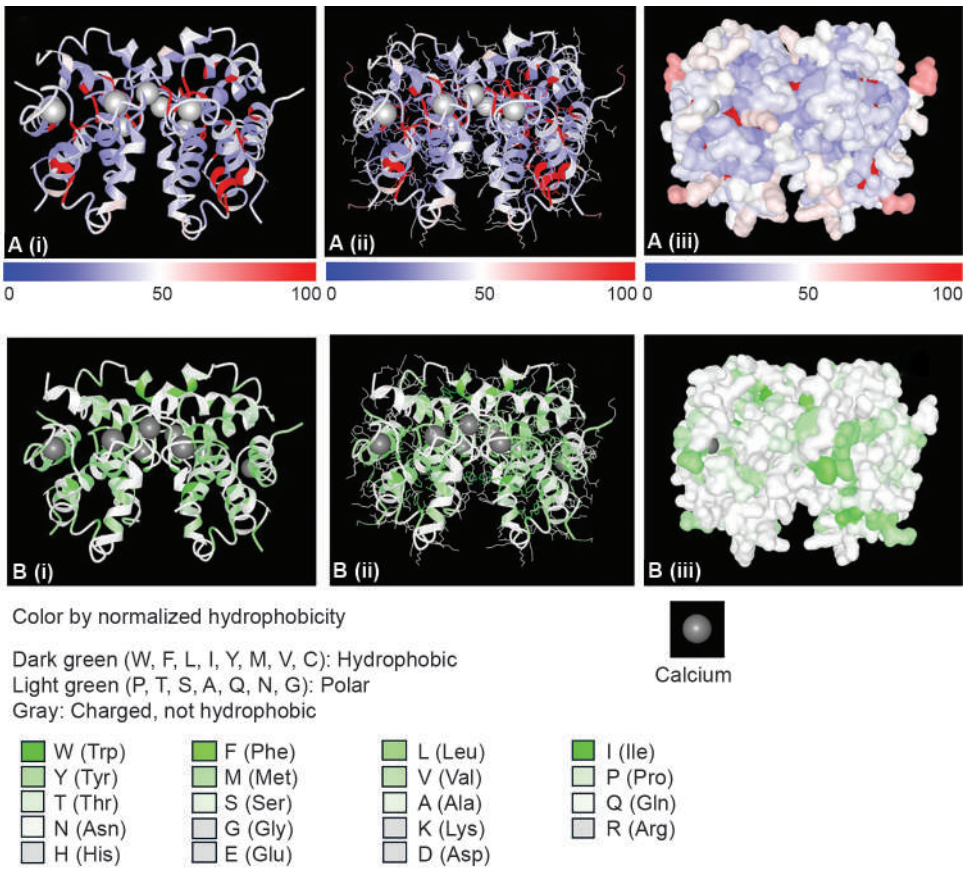
Source: The base structure was adopted from: https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html?&mmdbid=35490&bu=1&showanno=1&source=full-feature. This structure was derived from the iCn3D (short for "I see in 3D") format, a web-based interactive tool for analysis of three-dimensional macromolecular structures. This has been developed using the Web Graphics Library, a JavaScript Application Programming Interface to render 3D graphics in web browsers without requiring plugins. A JavaScript control and a shader code have been executed on the graphics processing unit for high-performance interactive graphics. These illustrations were prepared using data/tools provided on the web portals of the RCSB and NCBI PDBs, MMDB, GenBank, RefSeq, TPA, UniProtKB/Swiss-Prot database, PIR, PRF, and Microsoft PowerPoint, Microsoft Illustrator, and/or Adobe Photoshop

hence, the secretory mechanisms are largely non-classical and do not involve the conventional endoplasmic reticulum–Golgi pathway.[5,29] Calprotectin is released passively during cell death, especially during neutrophil necrosis or neutrophil extracellular trap (NET) formation, where it associates with DNA/histones into the extracellular space to trap microbes. The molecule is produced actively via cytoskeleton-dependent pathways involving membrane translocation and vesicle shedding.[62] In neutrophils, inflammatory activation mobilizes calprotectin to the cytosol and plasma membrane, followed by release through microvesicles or exocytosis-like processes.[63] During NET formation, calprotectin is expelled along with chromatin.[64] Once released, extracellular calprotectin contributes to a DAMP signal, amplifying inflammatory cascades by engaging pattern recognition receptors such as TLR4 and RAGE.[5] Levels of calprotectin, by whatever mechanism, show considerable variation between different individuals.

Host Defense

Calprotectin shows innate antimicrobial activity by at least three mechanisms:[5]

- Metal sequestration.
- Electrostatic interactions between its cationic surface regions and the anionic phospholipids in microbial membranes.[65] These interactions alter the local charge distribution, weakening lipid–lipid interactions and can reduce membrane stability, increase


A (i)
A (ii)
A (iii)
0
50
100
B (i)
B (ii)
B (iii)
Color by normalized hydrophobicity
Dark green (W, F, L, I, Y, M, V, C): Hydrophobic
Light green (P, T, S, A, Q, N, G): Polar
Gray: Charged, not hydrophobic
Calcium
W (Trp)
Y (Tyr)
T (Thr)
N (Asn)
H (His)
F (Phe)
M (Met)
S (Ser)
G (Gly)
E (Glu)
L (Leu)
V (Val)
A (Ala)
K (Lys)
D (Asp)
I (Ile)
P (Pro)
Q (Gln)
R (Arg)

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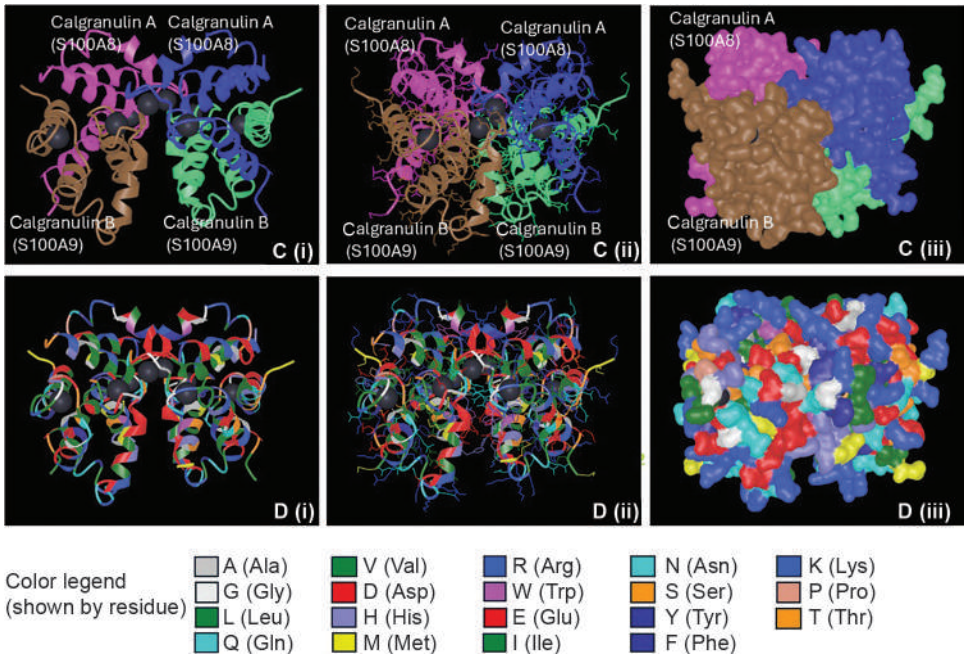


Figs 4A to D: In the presence of high concentrations of Ca^{++} ions, calprotectin heterodimers usually join to form heterotetramers composed of two *calgranulin A* (*S100A8*) and *calgranulin B* (*S100A9*) subunits. The illustrations here show the heterotetramers in the same sequence as in earlier figures. From left to right, we show: (i) primary ribbon structures with folds in the protein backbone; (ii) a structure with side chains; and (iii) the molecular surface. (A) Solvent accessibility. Color change from blue-to-white-to-red indicates increasing solvent accessibility. Value of ≥35% suggests that the protein surface is well-hydrated; (B) Hydrophobicity. Predicted solvent-accessible surface area (SASA), shown in a scale ranging from a deep green color at its low end to high white: (i–ii) primary ribbon structure and the structure with side-chains shows the green and white color residues as evenly distributed; and (iii) solvent-accessible space-filling model show a predominance of white surfaces, suggesting that hydrophobic green residues are buried in the core of the protein, Here, the SASA method shows that the protein surface is relatively solvent accessible; (C) Heterotetramers of two *calgranulin A* (*S100A8*) and *calgranulin B* (*S100A9*); (D) Amino acid composition of the calprotectin heterotetramer. The amino acid color-coding is shown below the figure

Source: The base structure was adopted from: https://www.ncbi.nlm.nih.gov/Structure/icn3d/full.html?&mmdbid=35490&bu=1&showanno=1&source=full-feature. This structure was derived from the iCn3D (short for "I see in 3D") format, a web-based interactive tool for analysis of three-dimensional macromolecular structures. This has been developed using the Web Graphics Library, a JavaScript Application Programming Interface to render 3D graphics in web browsers without requiring plugins. A JavaScript control and a shader code have been executed on the graphics processing unit for high-performance interactive graphics. These illustrations were prepared using data/tools provided on the web portals of the RCSB and NCBI PDBs, MMDB, GenBank, RefSeq, TPA, UniProtKB/Swiss-Prot database, PIR, PRF, and Microsoft PowerPoint. Microsoft Illustrator, and/or Adobe Photoshop

permeability, and compromise membrane-associated processes essential for microbial viability.[66]

- Hydrophobic interactions with microbial membranes, which can expose nonpolar surface residues associated with lipid acyl chains.[49] These hydrophobic contacts can possibly facilitate partial insertion of calprotectin into the phospholipid bilayer, altering lipid packing and membrane organization, and ultimately, disrupting the microbial membranes.[16]

These membrane changes can also enhance the bactericidal efficacy of innate immune cells by making pathogens more vulnerable to phagocytosis and intracellular killing.

Bacterial Molecular Targets

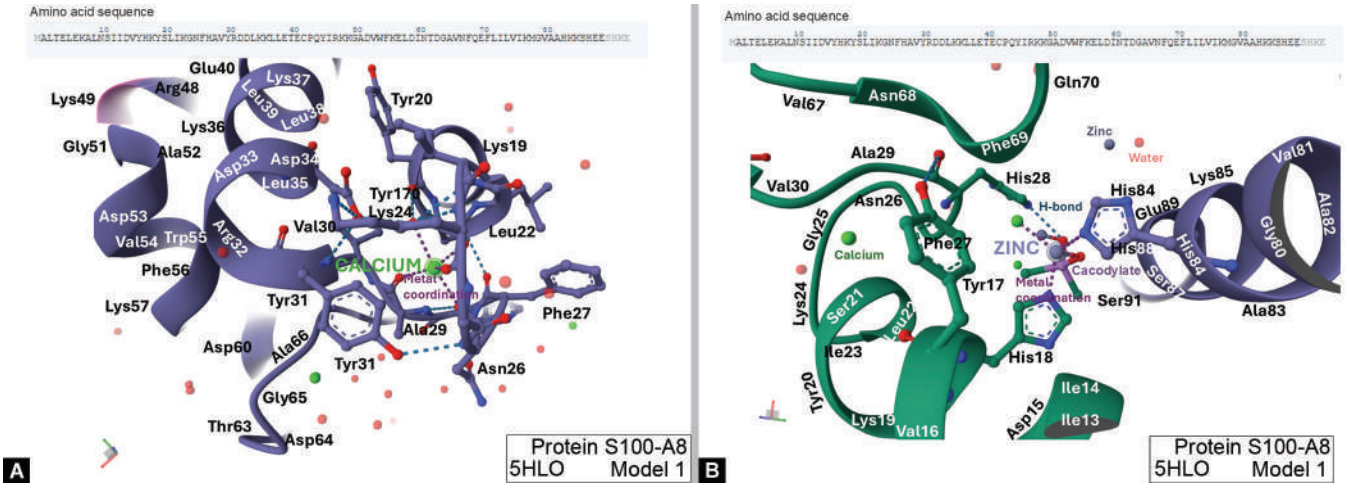
Calprotectin targets the following molecular systems in bacteria:

Zinc Uptake Systems

- Zinc uptake ATP-binding box complex (ZnuABC) transporter in gram-negative bacteria:[67] many gram-negative pathogens rely heavily on the ZnuABC transporter under Zn-depleted conditions; it is a high-efficiency bacterial Zn uptake regulator composed of a periplasmic binding protein (ZnuA), a transmembrane protein (ZnuB), and an ATPase (ZnuC).[68]
- Zinc uptake regulator (Zur) regulon is a set of bacterial genes, including ZnuABC, involved in high-affinity Zn acquisition.[69] It allows bacteria to partially overcome calprotectin-mediated metal starvation, maintaining essential enzymatic activities and supporting survival.
- Adenine complex ATP-binding cassette (AdcABC) system is a high-affinity Zn-uptake complex in *Streptococcus pneumoniae* (*S. pneumoniae*).[70] It is comprised of the AdcA Zn-binding lipoprotein with AdcB and AdcC permeases. When calprotectin causes Zn depletion, the bacterial AdcABC genes help maintain essential enzymatic functions and cellular processes.[71]

Manganese Uptake Systems

- Manganese transport protein H (MntH) is a proton-dependent divalent metal transporter of the natural resistance-associated macrophage protein (NRAMP) family; it helps bacteria overcome calprotectin-mediated metal starvation by importing Mn^{++} and some Fe^{++}.[72,73] The NRAMPs are evolutionarily conserved metal transporters found in bacteria, fungi, plants, and animals.



Figs 5A and B: Human *S100A8* (Protein databank entry 5HLO). (A) Calcium binding site. Ca^{++} binds to EF-hand motifs present in the *S100* subunits (*S100A*8 and *S100A9*). It induces conformational changes to promote dimer/tetramer formation; (B) A zinc-binding site containing several histidine residues in the vicinity. A cacodylate ion is also seen; it is a water-soluble organoarsenic compound [$(CH_3)_2AsO_2H$] of hitherto uncertain function. The term *S100* signifies that these proteins are soluble in 100% ammonium sulfate at neutral pH. The full name *S100A8* contains the serial number A8 of the protein in this series. Ala, Alanine; Arg, arginine; Asp, aspartate; Asn, asparagine; Glu, glutamate; Gly, glycine; H-bond, hydrogen bond; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Ser, serine; Tyr, tyrosine; Val, valine; 5HLO, crystal structure of calcium and zinc-bound human *S100A8* in space group $C222_1$. Based on the crystallography results, the space group (Hermann-Mauguin symbol) is labeled as $C222_1$ in a C-centered orthorhombic crystal system. There is a two-fold rotation axis along the crystallographic axes, and a third 2_1 screw axis is also seen

- Pneumococcal surface antigen B, C, and A (PsaBCA) transporter is a high-affinity Mn uptake system in *S. pneumoniae*.[74–76] The PsaABC is a crucial ATP-binding cassette comprised of PsaA (metal-binding lipoprotein), PsaB (ATPase), and PsaC (permease) components encoded within the psaBCA operon. It helps these bacteria salvage Mn^{++} from the extracellular environment; even trace amounts of Mn can help the bacteria maintain vital enzymatic functions, oxidative stress defense, and overall cellular homeostasis.[77]
- *Salmonella* iron transporter ABCD (SitABCD) is an ATP-binding cassette found in *Salmonella enterica* serovar Typhimurium and other pathogenic enterobacteria.[78,79] It promotes ATP hydrolysis-driven transport of substrates across biological membranes. Despite its name labeling it as an iron transporter, it is primarily a Mn^{++} transporter that can transport some iron (Fe^{++}) and help these bacteria survive in calprotectin-rich environments.[80] When extracellular Mn is depleted by calprotectin, bacteria upregulate SitABCD expression, enabling efficient scavenging of trace Mn from the host environment.[81] This allows essential enzymatic processes, oxidative stress defense, and cellular metabolism to continue despite metal restriction.

Many pathogens combine metal uptake with efflux systems to fine-tune intracellular levels of Zn and Mn, avoiding both starvation and toxicity.

- Bacteria can upregulate high-affinity metal uptake systems, such as ZnuABC, PsaBCA, SitABCD, and AdcABC, and also coordinate metal efflux systems such as cation diffusion facilitators, resistance-nodulation-cell division transporters, and P-type ATPases to maintain optimal intracellular metal concentrations.[82] In *S. pneumoniae*, the zinc effluxer cadmium, cobalt, and zinc/H^+-K^+ antiporter (CzcD) is upregulated during zinc stress.[83] This dual strategy allows these pathogens to efficiently scavenge trace metals from the environment.
- Some pathogens can sequester Zn and Mn by expressing zincophore or metallophore proteins.[84,85] These small, high-affinity molecules chelate trace metals from the host environment, effectively competing with calprotectin and other host proteins for essential nutrients.[86] The Zn-binding siderophores, the zincophores, chelate zinc in a host microenvironment.[87] Zinc is an essential micronutrient required for numerous bacterial enzymes and regulatory proteins, but its availability is restricted during infection by host metal-sequestering proteins such as calprotectin.[88] The best known zincophores include staphylopine in *Staphylococcus aureus* and pseudopaline in *Pseudomonas aeruginosa*; these molecules belong to a broader family of metallophores capable of binding multiple divalent metals, including Zn, Ni, and cobalt.[89–91] After chelating Zn, the zincophore–metal complex is transported into the bacterial cell.[92] These proteins play an important role in bacterial survival and virulence.
- Siderophores are small, high-affinity molecules secreted by bacteria, fungi, and some other microorganisms to scavenge iron from the environment, essential for their growth and metabolism.[93] In the host, iron with the host is tightly bound to proteins like transferrin, lactoferrin, and ferritin, making it largely inaccessible to invading pathogens.[94] Siderophores overcome this limitation by binding iron with extremely high affinity and transporting it back into microbial cells via specific receptors.[95] In host–pathogen interactions, siderophores play a critical role in microbial survival and virulence, and the host immune system counters them through nutritional immunity.[96] Proteins such as calprotectin and lipocalin-2 sequester metals or intercept siderophores, thereby limiting microbial proliferation.[20]

Genetics

Calprotectin mutations are uncommon; major mutations often lead to early resorption of the embryo.[97] Many single-nucleotide polymorphisms associated with altered inflammatory responses have been identified (Fig. 2).[17,18,98–100] Variations, including *S100A9* dimers and truncated forms, have been recognized.[101]

Epigenetics

Calprotectin isoforms and their expression patterns differ between neonates and adults, although the core heterodimer (*S100A8/S100A9*) is the same.[102] In the perinatal period, some post-translational modifications can alter stability, metal-binding capacity, and antimicrobial activity:[103]

- Phosphorylation:
  - *S100A9* is phosphorylated on serine and threonine residues, which can influence heterodimer stability, subcellular localization, and interactions with other proteins.[104]
  - Phosphorylation may enhance inflammatory activity and higher-order oligomers.[105]
- Oxidation:
  - Methionine and cysteine residues in both subunits can be oxidized.[106]
  - Oxidation can alter metal-binding, antimicrobial activity, and TLR4 activation.[107]
- Proteolytic processing:
  Partial proteolysis of *S100A8* or *S100A9* can generate fragments with altered chemotactic or antimicrobial activity.[9]
- Citrullination:
  Arginine to citrulline conversion can modulate inflammatory signaling.[108] These reactions usually involve enzymes known as peptidylarginine deiminases.[109]

These PTMs alter the capacities of calprotectin as an antimicrobial, metal-sequestering, and proinflammatory agent, depending on the tissue environment, oxidative stress, and developmental stage.

Evolution of Calprotectin

Calprotectin is a highly conserved component of the innate immune system. Similarly, a number of structural elements, such as the EF-hand Ca^{++}-binding motifs and high-affinity transition metal-binding sites, have been retained across the vertebrates.[5,21] There has been a strong evolutionary pressure to preserve its antimicrobial functions employing membrane destabilization and nutritional immunity (by sequestering local reserves of essential metals).[16,110–112] These non-specific defense mechanisms are crucial elements of vertebrate innate immunity.

About 500 million years ago, an ancestral protein with EF motifs developed in jawless fish (Agnatha).[113,114] This sequence then evolved into an *S100* gene with simple regulatory and structural features (Table 1).[115] Some of these *S100* genes differentiated into an *S100A* subfamily in cartilaginous fish via duplication events.[116] To recollect, a gene can evolve into (1) orthologous homologs with retained sequences and function over time in different species; and/or (2) paralogous genes that arise from duplication within a genome, producing copies that can diverge in sequence, regulation, or function over time.[117] Many *S100* paralogs can be seen in present-day cartilaginous fish (sharks and rays), bony fish, and amphibians.[116] These genes began to show functional diversification with specialized roles in innate immunity, inflammation, and cellular signaling.[118] It is difficult to establish a clear one-to-one ortholog relationship with all modern human *S100* genes, except for a few related to *S100B*, a crucial calcium-binding protein in the nervous system.[116] Later in evolution, the *S100A* subfamily grew further via gene duplication and pathogen-driven positive selection, and the paralogs underwent functional diversification for roles in innate immunity, inflammation, and cellular signaling.[119]

The *S100A8* (*calgranulin A*) and *S100A9* (*calgranulin B*) genes are believed to have evolved in mammals through duplication within the *S100* gene family, not from speciation.[120,121] Genomic, phylogenetic, and functional evidence suggest that these are paralogs, but pathogen-driven positive selection has shaped the regulatory regions and protein sequences.[118,122] The two proteins form a stable heterodimer, calprotectin; this co-dependence reflects coevolution after duplication.[61] Calprotectin is expressed by activated neutrophils, monocyte-derived/tissue-resident inflammatory macrophages, and other cells; the baseline expression is low in macrophages, but it is readily inducible during inflammation.[61]

In humans, *S100A8* and *S100A9* are located next to each other in the *S100* gene cluster on chromosome 1q21;[123,124] this chromosomal segment is known to have expanded through repeated tandem duplications and copy-number variation.[125,126] Other than the *S100* gene family, there are others such as the filaggrin/FLG-related genes and other epidermal differentiation and immune-related genes.[127,128] There is some supporting evidence of segmental duplications, repetitive elements, and recombination hotspots, which promote unequal crossing-over and recurrent duplication events.[129] This chromosome has played a major role in the expansion of gene families involved in immunity, inflammation, and barrier function, although this instability also makes it susceptible to deletions and duplications associated with disease.[130]

Even though *S100A8* and *S100A9* gene subsets are typically viewed as paralogs that arose from a duplication in an ancestral *S100A* gene early in mammalian evolution, we cannot confidently exclude the possibility that the *S100A9* gene may have appeared much later during evolution than *S100A8* (Fig. 6A).[45,131,132] The appearance of a paralogous proximity between the two could well have alternative explanations, such as sequence divergence events, gene conversion, and/or lineage-specific gene losses.[133–140] In addition, further rounds of duplication within the gene family can create paralogs of different evolutionary ages.[141] These paralogs could appear to have "evolved" at different time-points, when in reality their functional innovation or regulatory specialization happened long after their shared duplication event.[131,142] *S100A8* genes show more evolutionary divergence than *S100A9* (Figs 6B and C); the two genes likely experienced asymmetric evolutionary constraints after duplication, leading to different selective pressures.[120,143,144]

The evolutionary analysis shown above was developed using the software suite MEGA 12.[145] The phylogeny was inferred using the maximum likelihood method and the Jones–Taylor–Thornton (1992) model of amino acid substitutions, and the tree with the highest log likelihood (–9,220.17) is shown.[146] The initial tree for the heuristic search was selected by choosing one with superior log-likelihood from the Neighbor-Joining and maximum parsimony (MP) algorithms.[147] The chosen MP tree had the shortest length in

Table 1: Evolution of *S100A8* and *S100A9* genes

Evolutionary stage	*Timeline*	*Evolution of S100 genes*	*Evolution of S100A8/ S100A9 genes*	*Calprotectin (S100A8-S100A9 heterodimer)*	*Evolutionary events*
Early vertebrates (jawless fish; agnatha)	500 mya	Basic structure, ancestral EF hand proteins	None	None	
Cartilaginous fish (sharks, rays)	400 mya	Expanding groups of *S100* proteins	None	None	Some *S100* proteins now developing anti-microbial properties
Bony fish (teleosts)	380 mya	Expanding groups of *S100* proteins	Absent or distant homologs	None	
Amphibians and reptiles	320 mya	Expanding groups of *S100* proteins	Distant *S100A*-like genes	None	First signs of innate immunity
Early mammals	225 mya	Nearly full *S100A* family of proteins	*S100A8* and *S100A9* emerge via duplication *S100A8* and A100A9 dimers form	Present	Functional calprotectin heterodimer forms; innate immunity begins to mature
Primates and humans	55 mya	Conserved *S100A8/ S100A9*	Highly expressed, divergent *S100A8*	Present	*S100A8* diverges faster; *S100A9* more conserved; calprotectin develops in leukocytes and plays important antimicrobial and anti-inflammatory roles

Mya, million years ago

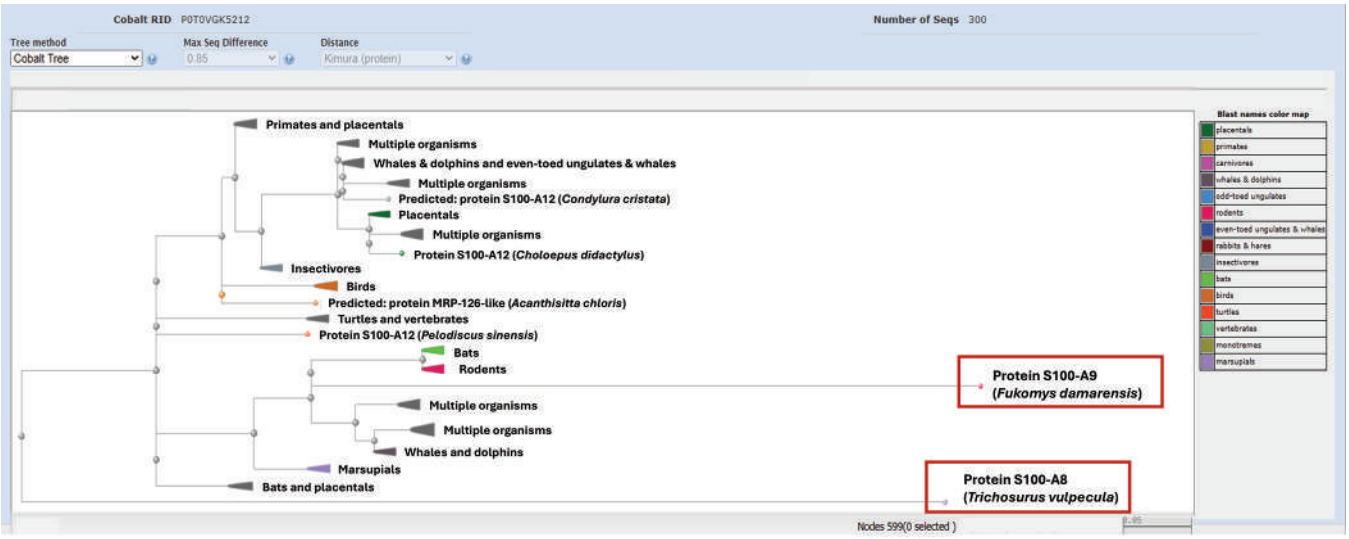
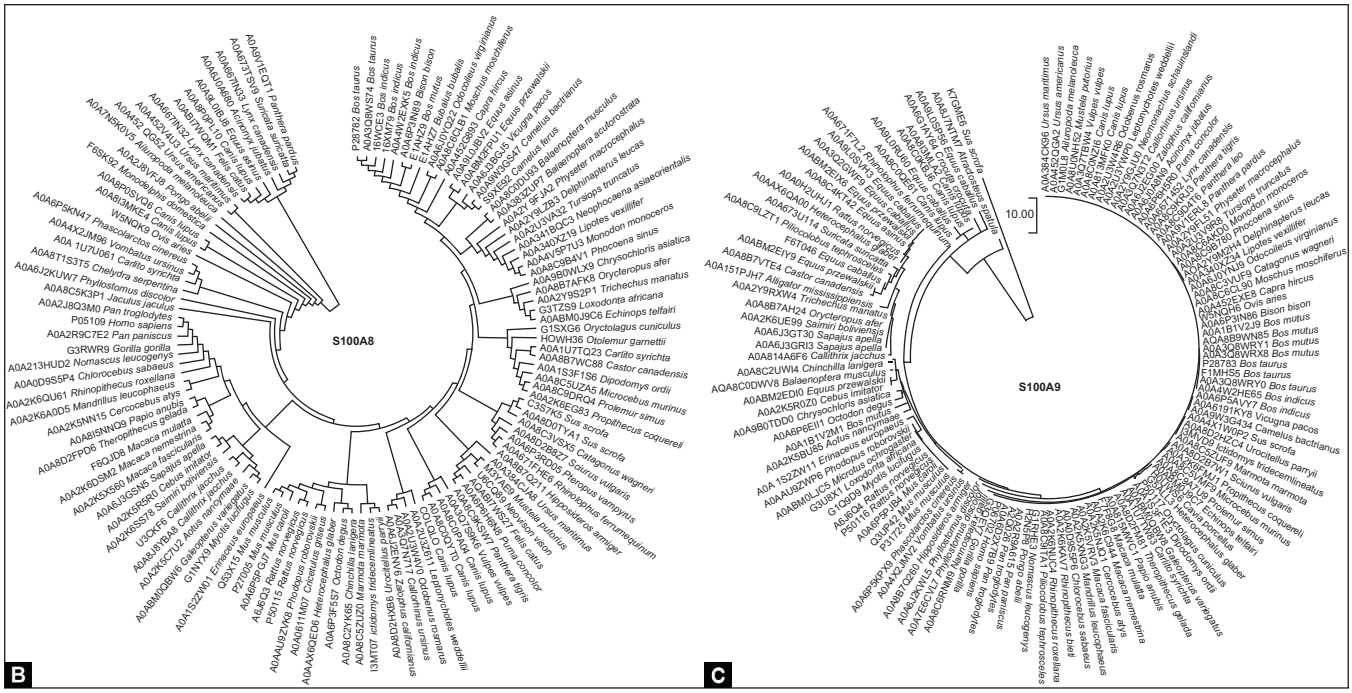


Fig. 6A: (A) Neighbor-joining (NJ) phylogenetic trees showing aerial views of the evolution of human *S100A8* and *S100A9*. These proteins each contain a calcium-binding site in the EF-hand motifs present in the *S100* subunits (*S100A8* and *S100A9*); calcium binding induces conformational changes to promote dimer/tetramer formation. A histidine-rich zinc-binding site is seen in the vicinity. There is also a cacodylate ion, a water-soluble organoarsenic compound [$(CH_3)_2AsO_2H$] of hitherto uncertain function. The NJ tree is an unrooted, distance-based depiction of iterative clustering of the most closely related taxa (species/sequences). This method minimizes the total branch length in estimating evolutionary divergence; the distance matrix is seen as input. There is some correction for unequal evolutionary rates. The branch lengths are proportional to evolutionary distance. *S100A8* was first seen in *Trichosurus vulpecula*, the common brushtail possum. It diverged in marsupials during the Pliocene era, around 5–2.6 million years ago. *S100A9* appeared more recently in *Fukomys damarensis*, the Damaraland mole-rat, within the last million years. The term *S100* signifies that these proteins are soluble in 100% ammonium sulfate at neutral pH. The full name *S100A8* contains the serial number A8 of the protein in this series. Ala, alanine; Arg, arginine; Asp, aspartate; Asn, asparagine; Glu, glutamate; Gly, glycine; H-bond, hydrogen bond; His, histidine; Ile, isoleucine; Leu, leucine; Lys, lysine; Phe, phenylalanine; Ser, serine; Tyr, tyrosine; Val, valine; 5HLO, crystal structure of calcium and zinc-bound human *S100A8* in space group $C222_1$. Based on the crystallography results, the space group (Hermann-Mauguin symbol) is labeled as $C222_1$ in a C-centered orthorhombic crystal system. There is a two-fold rotation axes along the crystallographic axes, and a third 21 screw axis is also seen. The phylogenetic tree was drawn using tools available on the TreeView site: https://www.ncbi.nlm.nih.gov/blast/treeview/treeView.cgi?request=page&cobaltRID=P0T0VGK5212&request=page&checkStatus=on&link_loc=alignPage&screenWidth=1536&screenHeight=864



Figs 6B and C: (B) Evolution of human *S100A8*: A detailed pie-chart phylogram is shown down to the species level, constructed with currently available data. The large number of branches could be an artifact arising from the inclusion of more taxa, but may also indicate rapid evolutionary splitting and adaptive radiation due to new ecological opportunities/environmental changes. Branch lengths are still an estimation of the evolutionary changes, but the tips/nodes simultaneously display both divergence and distribution patterns; (C) Evolution of human *S100A9*: Human *S100A8* and *S100A9* likely evolved through duplication and diversification in the vertebrate *S100* gene family (chromosome Iq21), which expanded under strong immune-related selective pressures. *S100A9* shows less evolutionary divergence than *S100A8*

10 searches, each of which was started with a randomly generated baseline. The analytical procedure encompassed 117 amino acid sequences with 299 positions in the final dataset.

The regulatory evolution of *S100A8* may also have contributed. There is more adaptive variability in promoter/enhancer regions and gene sequence, allowing amino-acid changes without compromising heterodimer formation.[98] These could provide some explanation for lineage-specific differences in expression timing and magnitude. Overall, *S100A8* has shown a flexible modulatory role and has heterodimerized with the *S100A9* genes that displayed a core structural and stabilizing role.[45] This coupling provided a strong purifying selection to preserve the newly evolved dimer, implying that changes in one copy must be compensated by changes in the other to preserve overall function.[148,149] After duplication, paralogs are known to diverge in sequence and regulation, but if these form heterodimers, multiprotein complexes, or participate in tightly linked pathways, mutations in one gene that affect binding, stability, or activity can create selective pressures on the other to adapt correspondingly.[140,150,151] This interdependent evolution ensures proper complex formation, activity, or regulation, allowing the paralogs to specialize or acquire new functions while maintaining essential interactions.[140] Coevolution is particularly common in immune proteins, signaling molecules, and structural partners, where coordinated changes are critical for biological function.[152]

The regulatory elements of *S100A8* may also have contributed to asymmetric evolutionary constraints, leading to different selective pressures. There is greater adaptive variability in promoter/enhancer regions and gene sequence, allowing amino-acid changes without compromising heterodimer formation.[153–155] Consequently, there have been more lineage-specific differences in expression timing and magnitude.[50,156] These show a flexible modulatory role and heterodimerize with the *S100A9* genes that displayed a core structural and stabilizing role.[45] This coupling has provided selection pressures to preserve the newly evolved dimer.[45,120]

In a panoramic view, *S100A8* may have been a mammal-specific innovation in leukocyte-based innate immunity.[153] It then heterodimerized with *S100A9* to form calprotectin and took on specialized roles in inflammation and antimicrobial defense. Over time, *S100A8* has adapted to host–pathogen interactions and undergone sequence and regulatory divergence. *S100A9* also arose in the *S100A* subfamily and contributed to innate immunity. It stabilizes the calprotectin heterodimer and contributes to metal-chelating antimicrobial activity.

There is a possibility of functional asymmetry that followed duplication in the two gene sets. *S100A8* has shown more amino-acid changes, particularly in surface-exposed and regulatory regions. There is also greater divergence in promoter and enhancer elements, leading to more lineage-specific expression patterns. *S100A9* has maintained core structural and functional roles in the calprotectin heterodimer, imposing stronger purifying selection. Compared to *S100A8*, *S100A9* has remained relatively conserved in sequence, reflecting strong purifying selection to maintain its structural and functional roles in inflammation. Recent data show that *S100A9* may exist in more than one isoform, one that is full-length and a shorter variant, *S100A9**, leading to different complex formations (heterodimers/tetramers) with *S100A8* and varied functions in inflammation and disease.[157]

Laboratory Measurement

Calprotectin is a useful biomarker of inflammation in biological fluids such as meconium, feces, serum, plasma, and other body fluids.[158,159] As a large protein complex, it does not readily move across anatomical boundaries. Immunoassay-based techniques, such as enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays, or fluorescence immunoassays, are used most frequently to measure the *S100A8/S100A9* heterodimer.[160] These assays show high sensitivity and specificity.[160] Fecal calprotectin concentrations are useful for estimating intestinal inflammation because the protein is fairly stable in stool.[161] Sample handling, extraction methods, and biological variability can influence results and need standardization. Measured calprotectin levels correlate with neutrophil activity and inflammatory burden, and are useful for confirming the presence of an inflammatory response, monitoring the change in severity, and response to treatment.[162]

Clinical Importance

Neonates typically have higher serum concentrations (0.79 ± 0.33 μg/mL) and fecal levels of calprotectin (0.34 ± 0.68 mg/gm stool) than those seen in adults; in adults, serum/stool levels of calprotectin are considered important as these rise with acute or chronic inflammation, infection, autoimmune disease, or malignancy.[163–166] Neonatal gut macrophages show a hyper-inflammatory profile and produce calprotectin (unpublished data).[167] Serum/fecal calprotectin levels are physiologically high in the immediate postnatal period, possibly due to immune activation, gut colonization, and perinatal adaptations.[168] Levels gradually decrease with age and stabilize as the immune system undergoes maturation.[168,169]

Neonates typically show high intestinal calprotectin levels.[165] Neonatal sepsis, necrotizing enterocolitis (NEC), hypoxic stress, and/or infection are associated with higher serum/fecal calprotectin levels, but interpretation can be challenging because of higher and variable baseline levels during this period.[170,171] Clinical confounders such as neutropenia and/or hyperglycemia can alter calprotectin expression.[172–175] Serial fecal calprotectin levels can help monitor the severity of illness in sepsis, NEC, postoperative ileus, in the postoperative period after surgery for congenital heart defects, and in some intestinal infections.[56,176–178] In infants with Hirschsprung disease, fecal calprotectin can help assess clostridial infections and resulting gut inflammation.[179,180] It may allow tracking of microbial activity/overgrowth, dysmotility, or evolving complications. A gradual decline may reflect improving gut function.[158] Inter-individual variability linked to the type of feedings (breast milk vs formula), mode of delivery, antibiotic use, and intestinal microbiota composition may limit its value.[56,181]

In one study of NEC, serum calprotectin levels rose from 0.79 ± 0.33 μg/mL in controls to 28.7 ± 1.85 μg/mL in stage II and 38.2 ± 9.67 μg/L in stage III NEC.[164] In another, serum calprotectin levels were recorded as 72.6 ± 10.6 mg/dL in controls and 241.1 ± 57.5 mg/dL in NEC.[163] A compact review of a few studies showed elevated serum/stool calprotectin levels in NEC.[182] A meta-analysis of 10 studies with 568 patients with NEC showed a pooled sensitivity, specificity, diagnostic odds ratio (DOR), and area under the curve (AUC) of 0.86 [95% confidence interval (CI): 0.80–0.91], 0.79 (95% CI: 0.75–0.83), 34.78 (95% CI: 15.30–79.07), and 0.92, respectively.[183] The pooled sensitivity, specificity, DOR, and AUC of subgroup analysis were 0.85 (95% CI: 0.79–0.90), 0.89 (95% CI: 0.85–0.92), 41.03 (95% CI: 16.87–99.78), and 0.92 for nine studies using ELISA; 0.85 (95% CI: 0.79–0.90), 0.89 (95% CI: 0.85–0.92), 42.08 (95% CI: 18.44–96.04), and 0.93 for six prospective studies; 0.91 (95% CI: 0.82–0.97), 0.93 (95% CI: 0.88–0.96), 69.51 (95% CI: 17.67–273.40), and 0.95 for four studies of preterm infants; and 0.86 (95% CI: 0.77–0.92), 0.94 (95% CI: 0.90–0.97), 53.23 (95% CI: 15.68–180.73), and 0.94 five studies that defined NEC as stage II or above.

Elevated fecal calprotectin can be identified in neonatal diarrhea, where it can help assess the severity of gut mucosal inflammation.[184] Such measures are sensitive but not specific. Elevated fecal calprotectin in an infant with diarrhea may also be the result of food protein-induced enterocolitis, cow's milk protein allergy, or bacterial enteritis.[101] Altered fecal calprotectin levels can support diagnosis, help assess disease severity, and response to treatment, but these should be interpreted alongside other clinical/laboratory findings.

Serum calprotectin levels in neonates have been studied as a potential biomarker for the detection of early sepsis, but diagnostic accuracy varies.[185] Some studies have found it less valuable than procalcitonin and C-reactive protein, whereas others show promising sensitivity and specificity for sepsis/NEC if specific cut-off values (such as >1.7 μg/mL or 1.2 ng/mL) are used.[178,186,187] There may be a need to monitor inter-assay consistency and assay kit performance.[188]

Serial fecal calprotectin levels can help monitor the severity of illness in neonatal sepsis, NEC, neonatal postoperative ileus, and some intestinal infections.[56,176–178] In infants with Hirschsprung disease, fecal calprotectin can help calibrate clostridial involvement and intestinal inflammation.[179] Monitoring changes in calprotectin over time can help assess changes in the severity of inflammation, microbial activity/overgrowth, dysmotility, or evolving complications, whereas a gradual decline may reflect improving gut function.[158] Unfortunately, the diagnostic value of calprotectin levels may be limited due to the wide inter-individual variability, type of feedings (breast milk vs formula), mode of delivery, antibiotic use, and intestinal microbiota composition.[56,181]

In infants with enterostomies, fecal calprotectin levels again require cautious interpretation as levels are often elevated independent of overt pathology.[12] Exposure of the intestinal mucosa to altered luminal flow, surgical inflammation, and changes in microbial composition can all increase calprotectin release from neutrophils and epithelial cells.[189] Consequently, absolute cutoff values are poorly defined; it is more useful as a longitudinal marker.[190] Rising levels may help identify inflammation, as in enterocolitis/infection, or in mucosal recovery prior to stoma closure.[158] Declining levels may indicate adaptation and healing.[158]

Conclusion

Calprotectin has been found of clinical value as a reliable, circulating biomarker of inflammation in the newborn. Elevated plasma levels reflect neutrophil-driven inflammation, making it particularly useful for detecting active disease, predicting relapses, and evaluating treatment effectiveness, thereby improving patient management. Fecal calprotectin is particularly useful in differentiating inflammatory from functional bowel disorders to inform responses to therapy. It is interesting to speculate on the therapeutic value of a multifunctional molecule of this nature. However, its biology suggests that the variability in its production and its low specificity are likely to limit its probable worth as a

synthetic therapy. Perhaps a focus on calprotectin's long-evolved powers to scavenge nutrients offers opportunities to manage invasive, resistant bacteria on the neonatal unit.

References

1. Lusitani D, Malawista SE, Montgomery RR. Calprotectin, an abundant cytosolic protein from human polymorphonuclear leukocytes, inhibits the growth of Borrelia burgdorferi. Infect Immun 2003;71(8):4711–4716. DOI: 10.1128/IAI.71.8.4711-4716.2003.
2. Murray J, Kok KB, Ayling RM. Fecal calprotectin in gastrointestinal disease. Clin Chem 2023;69(7):699–710. DOI: 10.1093/clinchem/hvad051.
3. Roseth AG, Fagerhol MK, Aadland E, et al. Assessment of the neutrophil dominating protein calprotectin in feces. A methodologic study. Scand J Gastroenterol 1992;27(9):793–798. DOI: 10.3109/00365529209011186.
4. Fukunaga S, Kuwaki K, Mitsuyama K, et al. Detection of calprotectin in inflammatory bowel disease: Fecal and serum levels and immunohistochemical localization. Int J Mol Med 2018;41(1):107–118. DOI: 10.3892/ijmm.2017.3244.
5. Jukic A, Bakiri L, Wagner EF, et al. Calprotectin: From biomarker to biological function. Gut 2021;70(10):1978–1988. DOI: 10.1136/gutjnl-2021-324855.
6. Inciarte-Mundo J, Frade-Sosa B, Sanmarti R. From bench to bedside: Calprotectin (S100A8/S100A9) as a biomarker in rheumatoid arthritis. Front Immunol 2022;13:1001025. DOI: 10.3389/fimmu.2022.1001025.
7. Nishimura F, Terranova VP, Sawa T, et al. Migration inhibitory factor related protein-8 (MRP-8) is an autocrine chemotactic factor for periodontal ligament cells. J Dent Res 1999;78(6):1251–1255. DOI: 10.1177/00220345990780060901.
8. Rammes A, Roth J, Goebeler M, et al. Myeloid-related protein (MRP) 8 and MRP14, calcium-binding proteins of the S100 family, are secreted by activated monocytes via a novel, tubulin-dependent pathway. J Biol Chem 1997;272(14):9496–9502. DOI: 10.1074/jbc.272.14.9496.
9. Wang S, Song R, Wang Z, et al. S100A8/A9 in Inflammation. Front Immunol 2018;9:1298. DOI: 10.3389/fimmu.2018.01298.
10. Moore BW. A soluble protein characteristic of the nervous system. Biochem Biophys Res Commun 1965;19(6):739–744. DOI: 10.1016/0006-291x(65)90320-7.
11. Baishya J, Everett JA, Chazin WJ, et al. The innate immune protein calprotectin interacts with and encases biofilm communities of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Front Cell Infect Microbiol 2022;12:898796. DOI: 10.3389/fcimb.2022.898796.
12. Kapel N, Ouni H, Benahmed NA, et al. Fecal calprotectin for the diagnosis and management of inflammatory bowel diseases. Clin Transl Gastroenterol 2023;14(9):e00617. DOI: 10.14309/ctg.0000000000000617.
13. Lewit-Bentley A, Rety S. EF-hand calcium-binding proteins. Curr Opin Struct Biol 2000;10(6):637–643. DOI: 10.1016/s0959-440x(00)00142-1.
14. Nakashige TG, Stephan JR, Cunden LS, et al. The hexahistidine motif of host-defense protein human calprotectin contributes to zinc withholding and its functional versatility. J Am Chem Soc 2016;138(37):12243–12251. DOI: 10.1021/jacs.6b06845.
15. Nelson MR, Chazin WJ. Structures of EF-hand Ca(2+)-binding proteins: Diversity in the organization, packing and response to Ca2+ binding. Biometals 1998;11(4):297–318. DOI: 10.1023/a:1009253808876.
16. Yousefi R, Imani M, Ardestani SK, et al. Human calprotectin: Effect of calcium and zinc on its secondary and tertiary structures, and role of pH in its thermal stability. Acta Biochim Biophys Sin (Shanghai) 2007;39(10):795–802. DOI: 10.1111/j.1745-7270.2007.00343.x.
17. Farag A, Shoaib M, Labeeb A, et al. S100A8 (rs3806232) gene polymorphism and S100A8 serum level in psoriasis vulgaris patients: A preliminary study. J Cosmet Dermatol 2022;21(10):4974–4982. DOI: 10.1111/jocd.14928.
18. Sun X, Meng H, Shi D, et al. Analysis of plasma calprotectin and polymorphisms of S100A8 in patients with aggressive periodontitis. J Periodontal Res 2011;46(3):354–360. DOI: 10.1111/j.1600-0765.2011.01350.x.
19. Ortega FJ, Mercader JM, Moreno-Navarrete JM, et al. Targeting the association of calgranulin B (S100A9) with insulin resistance and type 2 diabetes. J Mol Med (Berl) 2013;91(4):523–534. DOI: 10.1007/s00109-012-0979-8.
20. Zygiel EM, Nolan EM. Transition metal sequestration by the host-defense protein calprotectin. Annu Rev Biochem 2018;87:621–643. DOI: 10.1146/annurev-biochem-062917-012312.
21. Sejersen K, Eriksson MB, Larsson AO. Calprotectin as a biomarker for infectious diseases: A comparative review with conventional inflammatory markers. Int J Mol Sci 2025;26(13):6476. DOI: 10.3390/ijms26136476.
22. Ramasamy R, Yan SF, Herold K, et al. Receptor for advanced glycation end products: Fundamental roles in the inflammatory response: Winding the way to the pathogenesis of endothelial dysfunction and atherosclerosis. Ann N Y Acad Sci 2008;1126:7–13. DOI: 10.1196/annals.1433.056.
23. Ehrchen JM, Sunderkotter C, Foell D, et al. The endogenous toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. J Leukoc Biol 2009;86(3):557–566. DOI: 10.1189/jlb.1008647.
24. Garcia V, Perera YR, Chazin WJ. A structural perspective on calprotectin as a ligand of receptors mediating inflammation and potential drug target. Biomolecules 2022;12(4):519. DOI: 10.3390/biom12040519.
25. Yang D, Han Z, Oppenheim JJ. Alarmins and immunity. Immunol Rev 2017;280(1):41–56. DOI: 10.1111/imr.12577.
26. Roh JS, Sohn DH. Damage-associated molecular patterns in inflammatory diseases. Immune Netw 2018;18(4):e27. DOI: 10.4110/in.2018.18.e27.
27. Datta S, Rahman MA, Koka S, et al. High mobility group box 1 (HMGB1): Molecular signaling and potential therapeutic strategies. Cells 2024;13(23):1946. DOI: 10.3390/cells13231946.
28. Sarsu SB, Erbagci AB, Ulusal H, et al. The place of calprotectin, lactoferrin, and high-mobility group box 1 protein on diagnosis of acute appendicitis with children. Indian J Surg 2017;79(2):131–136. DOI: 10.1007/s12262-015-1441-2.
29. Nisapakultorn K, Ross KF, Herzberg MC. Calprotectin expression inhibits bacterial binding to mucosal epithelial cells. Infect Immun 2001;69(6):3692–3696. DOI: 10.1128/IAI.69.6.3692-3696.2001.
30. Rugtveit J, Fagerhol MK. Age-dependent variations in fecal calprotectin concentrations in children. J Pediatr Gastroenterol Nutr 2002;34(3):323–324; author reply 324–325. DOI: 10.1097/00005176-200203000-00022.
31. Avau B, Van Remoortel H, De Buck E. Translation and validation of PubMed and Embase search filters for identification of systematic reviews, intervention studies, and observational studies in the field of first aid. J Med Libr Assoc 2021;109(4):599–608. DOI: 10.5195/jmla.2021.1219.
32. Burnham JF. Scopus database: A review. Biomed Digit Libr 2006;3:1. DOI: 10.1186/1742-5581-3-1.
33. Shabani F, Farasat A, Mahdavi M, et al. Calprotectin (S100A8/S100A9): A key protein between inflammation and cancer. Inflamm Res 2018;67(10):801–812. DOI: 10.1007/s00011-018-1173-4.
34. Xu L, Tian X, Zhang W-B. Heterodimeric protein entangling motifs: Systematic discovery, feature analysis, and topology engineering. Chem Sci 2025;16(36):16690–16702. DOI: 10.1039/D5SC03953C.
35. Zhang P, Han J, Cieplak P, et al. Determining the atomic charge of calcium ion requires the information of its coordination geometry in an EF-hand motif. J Chem Phys 2021;154(12):124104. DOI: 10.1063/5.0037517.
36. Bagur R, Hajnoczky G. Intracellular $Ca(2^+)$ sensing: Its role in calcium homeostasis and signaling. Mol Cell 2017;66(6):780–788. DOI: 10.1016/j.molcel.2017.05.028.
37. Yammani RR. S100 proteins in cartilage: Role in arthritis. Biochim Biophys Acta 2012;1822(4):600–606. DOI: 10.1016/j.bbadis.2012.01.006.
38. Denessiouk K, Permyakov S, Denesyuk A, et al. Two structural motifs within canonical EF-hand calcium-binding domains identify

five different classes of calcium buffers and sensors. PLoS One 2014;9(10):e109287. DOI: 10.1371/journal.pone.0109287.
39. Dale I, Fagerhol MK, Naesgaard I. Purification and partial characterization of a highly immunogenic human leukocyte protein, the L1 antigen. Eur J Biochem 1983;134(1):1–6. DOI: 10.1111/j.1432-1033.1983.tb07522.x.
40. Odink K, Cerletti N, Bruggen J, et al. Two calcium-binding proteins in infiltrate macrophages of rheumatoid arthritis. Nature 1987;330(6143):80–82. DOI: 10.1038/330080a0.
41. Teigelkamp S, Bhardwaj RS, Roth J, et al. Calcium-dependent complex assembly of the myeloic differentiation proteins MRP-8 and MRP-14. J Biol Chem 1991;266(20):13462–13467. PMID: 2071612.
42. Hessian PA, Edgeworth J, Hogg N. MRP-8 and MRP-14, two abundant Ca(2+)-binding proteins of neutrophils and monocytes. J Leukoc Biol 1993;53(2):197–204. PMID: 8445331.
43. Veiga A, Silva IV, Duarte MM, et al. Current trends on protein driven bioinks for 3D printing. Pharmaceutics 2021;13(9):1444. DOI: 10.3390/pharmaceutics13091444.
44. Hayden JA, Brophy MB, Cunden LS, et al. High-affinity manganese coordination by human calprotectin is calcium-dependent and requires the histidine-rich site formed at the dimer interface. J Am Chem Soc 2013;135(2):775–787. DOI: 10.1021/ja3096416.
45. Vogl T, Gharibyan AL, Morozova-Roche LA. Pro-inflammatory S100A8 and S100A9 proteins: Self-assembly into multifunctional native and amyloid complexes. Int J Mol Sci 2012;13(3):2893–2917. DOI: 10.3390/ijms13032893.
46. Champaiboon C, Sappington KJ, Guenther BD, et al. Calprotectin S100A9 calcium-binding loops I and II are essential for keratinocyte resistance to bacterial invasion. J Biol Chem 2009;284(11):7078–7090. DOI: 10.1074/jbc.M806605200.
47. Brophy MB, Hayden JA, Nolan EM. Calcium ion gradients modulate the zinc affinity and antibacterial activity of human calprotectin. J Am Chem Soc 2012;134(43):18089–18100. DOI: 10.1021/ja307974e.
48. Silvers R, Stephan JR, Griffin RG, et al. Molecular basis of Ca(II)-induced tetramerization and transition-metal sequestration in human calprotectin. J Am Chem Soc 2021;143(43):18073–18090. DOI: 10.1021/jacs.1c06402.
49. Yousefi R, Ardestani SK, Saboury AA, et al. Investigation on the surface hydrophobicity and aggregation kinetics of human calprotectin in the presence of calcium. J Biochem Mol Biol 2005;38(4):407–413. DOI: 10.5483/bmbrep.2005.38.4.407.
50. Averill MM, Barnhart S, Becker L, et al. S100A9 differentially modifies phenotypic states of neutrophils, macrophages, and dendritic cells: Implications for atherosclerosis and adipose tissue inflammation. Circulation 2011;123(11):1216–1226. DOI: 10.1161/CIRCULATIONAHA.110.985523.
51. Kozlyuk N, Monteith AJ, Garcia V, et al. S100 proteins in the innate immune response to pathogens. Methods Mol Biol 2019;1929:275–290. DOI: 10.1007/978-1-4939-9030-6_18.
52. van Lent PLEM, Grevers L, Blom AB, et al. Myeloid-related proteins S100A8/S100A9 regulate joint inflammation and cartilage destruction during antigen-induced arthritis. Ann Rheum Dis 2008;67(12):1750–1758. DOI: 10.1136/ard.2007.077800.
53. Pruenster M, Vogl T, Roth J, et al. S100A8/A9: From basic science to clinical application. Pharmacol Ther 2016;167:120–131. DOI: 10.1016/j.pharmthera.2016.07.015.
54. Damo SM, Kehl-Fie TE, Sugitani N, et al. Molecular basis for manganese sequestration by calprotectin and roles in the innate immune response to invading bacterial pathogens. Proc Natl Acad Sci U S A 2013;110(10):3841–3846. DOI: 10.1073/pnas.1220341110.
55. Li W, Chen Q, Peng C, et al. Roles of the receptor for advanced glycation end products and its ligands in the pathogenesis of Alzheimer's disease. Int J Mol Sci 2025;26(1):403. DOI: 10.3390/ijms26010403.
56. Park JS, Cho JY, Chung C, et al. Dynamic changes of fecal calprotectin and related clinical factors in neonates. Front Pediatr 2020;8:326. DOI: 10.3389/fped.2020.00326.
57. Prins D, Arias CG, Klampfl T, et al. Mutant calreticulin in the myeloproliferative neoplasms. Hemasphere 2020;4(1):e333. DOI: 10.1097/HS9.0000000000000333.
58. Zygiel EM, Nolan EM. Exploring iron withholding by the innate immune protein human calprotectin. Acc Chem Res 2019;52(8):2301–2308. DOI: 10.1021/acs.accounts.9b00250.
59. Rosen T, Wang KKA, Nolan EM. Metal sequestration by S100 proteins in chemically diverse environments. Trends Microbiol 2022;30(7):654–664. DOI: 10.1016/j.tim.2021.12.006.
60. Adhikari J, Stephan JR, Rempel DL, et al. Calcium binding to the innate immune protein human calprotectin revealed by integrated mass spectrometry. J Am Chem Soc 2020;142(31):13372–13383. DOI: 10.1021/jacs.9b11950.
61. Carnazzo V, Redi S, Basile V, et al. Calprotectin: Two sides of the same coin. Rheumatology (Oxford) 2024;63(1):26–33. DOI: 10.1093/rheumatology/kead405.
62. Voganatsi A, Panyutich A, Miyasaki KT, et al. Mechanism of extracellular release of human neutrophil calprotectin complex. J Leukoc Biol 2001;70(1):130–134. PMID: 11435495.
63. Yui S, Nakatani Y, Mikami M. Calprotectin (S100A8/S100A9), an inflammatory protein complex from neutrophils with a broad apoptosis-inducing activity. Biol Pharm Bull 2003;26(6):753–760. DOI: 10.1248/bpb.26.753.
64. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against Candida albicans. PLoS Pathog 2009;5(10):e1000639. DOI: 10.1371/journal.ppat.1000639.
65. Gheibi N, Ghorbani M, Shariatifar H, et al. In silico assessment of human Calprotectin subunits (S100A8/A9) in presence of sodium and calcium ions using molecular dynamics simulation approach. PLoS One 2019;14(10):e0224095. DOI: 10.1371/journal.pone.0224095.
66. Weise CF, Login FH, Ho O, et al. Negatively charged lipid membranes promote a disorder-order transition in the Yersinia YscU protein. Biophys J 2014;107(8):1950–1961. DOI: 10.1016/j.bpj.2014.09.005.
67. Santos RERS, da Silva Junior WP, Harrison S, et al. The zinc transporter ZnuABC is critical for the virulence of chromobacterium violaceum and contributes to diverse zinc-dependent physiological processes. Infect Immun 2021;89(11):e0031121. DOI: 10.1128/IAI.00311-21.
68. Burcham LR, Le Breton Y, Radin JN, et al. Identification of zinc-dependent mechanisms used by group B *Streptococcus* to overcome calprotectin-mediated stress. mBio 2020;11(6). DOI: 10.1128/mBio.02302-20.
69. Wang J, Lonergan ZR, Gonzalez-Gutierrez G, et al. Multi-metal restriction by calprotectin impacts de novo flavin biosynthesis in *Acinetobacter baumannii*. Cell Chem Biol 2019;26(5):745–755.e7. DOI: 10.1016/j.chembiol.2019.02.011.
70. Mikhaylina A, Ksibe AZ, Scanlan DJ, et al. Bacterial zinc uptake regulator proteins and their regulons. Biochem Soc Trans 2018;46(4):983–1001. DOI: 10.1042/BST20170228.
71. Ganguly T, Peterson AM, Kajfasz JK, et al. Zinc import mediated by AdcABC is critical for colonization of the dental biofilm by Streptococcus mutans in an animal model. Mol Oral Microbiol 2021;36(3):214–224. DOI: 10.1111/omi.12337.
72. Wu J, McAuliffe O, O'Byrne CP. Manganese uptake mediated by the NRAMP-type transporter MntH is required for acid tolerance in *Listeria monocytogenes*. Int J Food Microbiol 2023;399:110238. DOI: 10.1016/j.ijfoodmicro.2023.110238.
73. Bozzi AT, Gaudet R. Molecular mechanism of NRAMP-family transition metal transport. J Mol Biol 2021;433(16):166991. DOI: 10.1016/j.jmb.2021.166991.
74. Davidson AL, Dassa E, Orelle C, et al. Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbiol Mol Biol Rev 2008;72(2):317–364, table of contents. DOI: 10.1128/MMBR.00031-07.
75. Rajam G, Anderton JM, Carlone GM, et al. Pneumococcal surface adhesin A (PsaA): A review. Crit Rev Microbiol 2008;34(3–4):131–142. DOI: 10.1080/10408410802275352.

76. Kohler S, Voß F, Mejia AG, et al. Pneumococcal lipoproteins involved in bacterial fitness, virulence, and immune evasion. FEBS Lett 2016;590(21):3820–3839. DOI: 10.1002/1873-3468.12352.
77. Eijkelkamp BA, McDevitt CA, Kitten T. Manganese uptake and streptococcal virulence. Biometals 2015;28(3):491–508. DOI: 10.1007/s10534-015-9826-z.
78. Janakiraman A, Slauch JM. The putative iron transport system SitABCD encoded on SPI1 is required for full virulence of *Salmonella typhimurium*. Mol Microbiol 2000;35(5):1146–1155. DOI: 10.1046/j.1365-2958.2000.01783.x.
79. Nakashige TG, Zhang B, Krebs C, et al. Human calprotectin is an iron-sequestering host-defense protein. Nat Chem Biol 2015;11(10):765–771. DOI: 10.1038/nchembio.1891.
80. Zhou D, Hardt WD, Galan JE. Salmonella typhimurium encodes a putative iron transport system within the centisome 63 pathogenicity island. Infect Immun 1999;67(4):1974–1981. DOI: 10.1128/IAI.67.4.1974-1981.1999.
81. Kelliher JL, Kehl-Fie TE. Competition for manganese at the host-pathogen interface. Prog Mol Biol Transl Sci 2016;142:1–25. DOI: 10.1016/bs.pmbts.2016.05.002.
82. Ammendola S, Pasquali P, Pistoia C, et al. High-affinity Zn2+ uptake system ZnuABC is required for bacterial zinc homeostasis in intracellular environments and contributes to the virulence of *Salmonella enterica*. Infect Immun 2007;75(12):5867–5876. DOI: 10.1128/IAI.00559-07.
83. Kloosterman TG, van der Kooi-Pol MM, Bijlsma JJE, et al. The novel transcriptional regulator SczA mediates protection against Zn^{2+} stress by activation of the Zn^{2+}-resistance gene czcD in *Streptococcus pneumoniae*. Mol Microbiol 2007;65(4):1049–1063. DOI: 10.1111/j.1365-2958.2007.05849.x.
84. Rosen T, Hadley RC, Bozzi AT, et al. Zinc sequestration by human calprotectin facilitates manganese binding to the bacterial solute-binding proteins PsaA and MntC. Metallomics 2022;14(2):mfac001. DOI: 10.1093/mtomcs/mfac001.
85. Besold AN, Gilston BA, Radin JN, et al. Role of Calprotectin in withholding zinc and copper from *Candida albicans*. Infect Immun 2018;86(2). DOI: 10.1128/IAI.00779-17.
86. Arnold E. Non-classical roles of bacterial siderophores in pathogenesis. Front Cell Infect Microbiol 2024;14:1465719. DOI: 10.3389/fcimb.2024.1465719.
87. Holden VI, Bachman MA. Diverging roles of bacterial siderophores during infection. Metallomics 2015;7(6):986–995. DOI: 10.1039/c4mt00333k.
88. Maywald M, Rink L. Zinc in human health and infectious diseases. Biomolecules 2022;12(12). DOI: 10.3390/biom12121748.
89. Song L, Zhang Y, Chen W, et al. Mechanistic insights into staphylopine-mediated metal acquisition. Proc Natl Acad Sci U S A 2018;115(15):3942–3947. DOI: 10.1073/pnas.1718382115.
90. Ghssein G, Ezzeddine Z. A review of *Pseudomonas aeruginosa* metallophores: Pyoverdine, pyochelin and pseudopaline. Biology (Basel) 2022;11(12):1711. DOI: 10.3390/biology11121711.
91. Gomes AFR, Almeida MC, Sousa E, et al. Siderophores and metallophores: Metal complexation weapons to fight environmental pollution. Sci Total Environ 2024;932:173044. DOI: 10.1016/j.scitotenv.2024.173044.
92. Bellotti D, Rowinska-Zyrek M, Remelli M. How Zinc-binding systems, expressed by human pathogens, acquire zinc from the colonized host environment: A critical review on zincophores. Curr Med Chem 2021;28(35):7312–7338. DOI: 10.2174/1389200222666210514012945.
93. Ahmed E, Holmstrom SJM. Siderophores in environmental research: Roles and applications. Microb Biotechnol 2014;7(3):196–208. DOI: 10.1111/1751-7915.12117.
94. Ullah I, Lang M. Key players in the regulation of iron homeostasis at the host-pathogen interface. Front Immunol 2023;14:1279826. DOI: 10.3389/fimmu.2023.1279826.
95. Lan P, Lu Y, Fu Y, et al. Siderophores and beyond: A comprehensive review of iron acquisition in *Klebsiella pneumoniae*. Virulence 2025;16(1):2550621. DOI: 10.1080/21505594.2025.2550621.
96. Oliveira F, Lima T, Correia A, et al. Siderophore-mediated iron acquisition plays a critical role in biofilm formation and survival of *Staphylococcus epidermidis* within the host. Front Med (Lausanne) 2021;8:799227. DOI: 10.3389/fmed.2021.799227.
97. Passey RJ, Williams E, Lichanska AM, et al. A null mutation in the inflammation-associated S100 protein S100A8 causes early resorption of the mouse embryo. J Immunol 1999;163(4):2209–2216. PMID: 10438963.
98. Sun Y, Xu H, Gao W, et al. S100a8/A9 proteins: Critical regulators of inflammation in cardiovascular diseases. Front Cardiovasc Med 2024;11:1394137. DOI: 10.3389/fcvm.2024.1394137.
99. Li QY, Meng HX, Zhang L, et al. Correlation between single nucleotide polymorphisms in a calprotectin subunit gene and risk of periodontitis in a Chinese population. Ann Hum Genet 2007;71(Pt 3):312–324. DOI: 10.1111/j.1469-1809.2006.00326.x.
100. Farag AGA, Helal SG, Labib AZ, et al. Study of calprotectin gene polymorphism and serum level in acne vulgaris patients. Int J Dermatol 2022;61(10):1262–1269. DOI: 10.1111/ijd.16268.
101. Al-Beltagi M, Saeed NK, Bediwy AS, et al. Fecal calprotectin in pediatric gastrointestinal diseases: Pros and cons. World J Clin Pediatr 2024;13(2):93341. DOI: 10.5409/wjcp.v13.i2.93341.
102. Rodriguez-Belvis MV, Bris JFV, Fernandez CP, et al. Normal fecal calprotectin levels in healthy children are higher than in adults and decrease with age. Paediatr Child Health 2020;25(5):286–292. DOI: 10.1093/pch/pxz070.
103. Nolan EM, Peet JJY. Post-translational modifications on the metal-sequestering protein calprotectin. Biometals 2023;36(4):817–828. DOI: 10.1007/s10534-023-00493-x.
104. Bah I, Youssef D, Yao ZQ, et al. Hotairm1 controls S100A9 protein phosphorylation in myeloid-derived suppressor cells during sepsis. J Clin Cell Immunol 2023;14(4):1000691. PMID: 39665047.
105. Schenten V, Plancon S, Jung N, et al. Secretion of the phosphorylated form of S100A9 from neutrophils is essential for the proinflammatory functions of extracellular S100A8/A9. Front Immunol 2018;9:447. DOI: 10.3389/fimmu.2018.00447.
106. Magon NJ, Turner R, Gearry RB, et al. Oxidation of calprotectin by hypochlorous acid prevents chelation of essential metal ions and allows bacterial growth: Relevance to infections in cystic fibrosis. Free Radic Biol Med 2015;86:133–144. DOI: 10.1016/j.freeradbiomed.2015.05.022.
107. Hoskin TS, Crowther JM, Cheung J, et al. Oxidative cross-linking of calprotectin occurs in vivo, altering its structure and susceptibility to proteolysis. Redox Biol 2019;24:101202. DOI: 10.1016/j.redox.2019.101202.
108. Ciesielski O, Biesiekierska M, Panthu B, et al. Citrullination in the pathology of inflammatory and autoimmune disorders: Recent advances and future perspectives. Cell Mol Life Sci 2022;79(2):94. DOI: 10.1007/s00018-022-04126-3.
109. Kijak-Bockowska M, Czerwinska J, Owczarczyk-Saczonek A. Peptidylarginine deiminases: An overview of recent advances in citrullination research. Int J Mol Sci 2025;26(24):12060. DOI: 10.3390/ijms262412060.
110. Johnstone KF, Herzberg MC. Antimicrobial peptides: Defending the mucosal epithelial barrier. Front Oral Health 2022;3:958480. DOI: 10.3389/froh.2022.958480.
111. Kehl-Fie TE, Chitayat S, Hood MI, et al. Nutrient metal sequestration by calprotectin inhibits bacterial superoxide defense, enhancing neutrophil killing of Staphylococcus aureus. Cell Host Microbe 2011;10(2):158–164. DOI: 10.1016/j.chom.2011.07.004.
112. Weinberg ED. Iron availability and infection. Biochim Biophys Acta 2009;1790(7):600–605. DOI: 10.1016/j.bbagen.2008.07.002.
113. Schaub MC, Heizmann CW. Calcium, troponin, calmodulin, S100 proteins: From myocardial basics to new therapeutic strategies. Biochem Biophys Res Commun 2008;369(1):247–264. DOI: 10.1016/j.bbrc.2007.10.082.
114. DeLaurier A. Evolution and development of the fish jaw skeleton. Wiley Interdiscip Rev Dev Biol 2019;8(2):e337. DOI: 10.1002/wdev.337.

115. Donato R, Cannon BR, Sorci G, et al. Functions of S100 proteins. Curr Mol Med 2013;13(1):24–57. PMID: 22834835.
116. Kraemer AM, Saraiva LR, Korsching SI. Structural and functional diversification in the teleost S100 family of calcium-binding proteins. BMC Evol Biol 2008;8:48. DOI: 10.1186/1471-2148-8-48.
117. Koonin EV. Orthologs, paralogs, and evolutionary genomics. Annu Rev Genet 2005;39:309–338. DOI: 10.1146/annurev.genet.39.073003.114725.
118. Singh P, Ali SA. Multifunctional role of S100 protein family in the immune system: An update. Cells 2022;11(15):2274. DOI: 10.3390/cells11152274.
119. Marenholz I, Heizmann CW, Fritz G. S100 proteins in mouse and man: From evolution to function and pathology (including an update of the nomenclature). Biochem Biophys Res Commun 2004;322(4):1111–1122. DOI: 10.1016/j.bbrc.2004.07.096.
120. Harman JL, Loes AN, Warren GD, et al. Evolution of multifunctionality through a pleiotropic substitution in the innate immune protein S100A9. Elife 2020;9:e54100. DOI: 10.7554/eLife.54100.
121. Foell D, Wittkowski H, Vogl T, et al. S100 proteins expressed in phagocytes: A novel group of damage-associated molecular pattern molecules. J Leukoc Biol 2007;81(1):28–37. DOI: 10.1189/jlb.0306170.
122. Gonzalez LL, Garrie K, Turner MD. Role of S100 proteins in health and disease. Biochim Biophys Acta Mol Cell Res 2020;1867(6):118677. DOI: 10.1016/j.bbamcr.2020.118677.
123. Chen Y, Ouyang Y, Li Z, et al. S100A8 and S100A9 in cancer. Biochim Biophys Acta Rev Cancer 2023;1878(3):188891. DOI: 10.1016/j.bbcan.2023.188891.
124. Ridinger K, Ilg EC, Niggli FK, et al. Clustered organization of S100 genes in human and mouse. Biochim Biophys Acta 1998;1448(2):254–263. DOI: 10.1016/s0167-4889(98)00137-2.
125. Bernier R, Steinman KJ, Reilly B, et al. Clinical phenotype of the recurrent 1q21.1 copy-number variant. Genet Med 2016;18(4):341–349. DOI: 10.1038/gim.2015.78.
126. Vollger MR, Guitart X, Dishuck PC, et al. Segmental duplications and their variation in a complete human genome. Science 2022;376(6588):eabj6965. DOI: 10.1126/science.abj6965.
127. Romero V, Hosomichi K, Nakaoka H, et al. Structure and evolution of the filaggrin gene repeated region in primates. BMC Evol Biol 2017;17(1):10. DOI: 10.1186/s12862-016-0851-5.
128. Volz A, Korge BP, Compton JG, et al. Physical mapping of a functional cluster of epidermal differentiation genes on chromosome 1q21. Genomics 1993;18(1):92–99. DOI: 10.1006/geno.1993.1430.
129. Pang H, Yu X, Kim YM, et al. Disorders associated with diverse, recurrent deletions and duplications at 1q21.1. Front Genet 2020;11:577. DOI: 10.3389/fgene.2020.00577.
130. Hawerkamp HC, Fahy CMR, Fallon PG, et al. Break on through: The role of innate immunity and barrier defence in atopic dermatitis and psoriasis. Skin Health Dis 2022;2(2):e99. DOI: 10.1002/ski2.99.
131. Studer RA, Robinson-Rechavi M. How confident can we be that orthologs are similar, but paralogs differ? Trends Genet 2009;25(5):210–216. DOI: 10.1016/j.tig.2009.03.004.
132. Altenhoff AM, Studer RA, Robinson-Rechavi M, et al. Resolving the ortholog conjecture: Orthologs tend to be weakly, but significantly, more similar in function than paralogs. PLoS Comput Biol 2012;8(5):e1002514. DOI: 10.1371/journal.pcbi.1002514.
133. Gout JF, Kahn D, Duret L, et al. The relationship among gene expression, the evolution of gene dosage, and the rate of protein evolution. PLoS Genet 2010;6(5):e1000944. DOI: 10.1371/journal.pgen.1000944.
134. Torres EM, Williams BR, Amon A. Aneuploidy: Cells losing their balance. Genetics 2008;179(2):737–746. DOI: 10.1534/genetics.108.090878.
135. Payer B, Lee JT. X chromosome dosage compensation: How mammals keep the balance. Annu Rev Genet 2008;42:733–772. DOI: 10.1146/annurev.genet.42.110807.091711.
136. Wolfe KH. Yesterday's polyploids and the mystery of diploidization. Nat Rev Genet 2001;2(5):333–341. DOI: 10.1038/35072009.
137. Makowsky R, Cox CL, Roelke C, et al. Analyzing the relationship between sequence divergence and nodal support using Bayesian phylogenetic analyses. Mol Phylogenet Evol 2010;57(2):485–494. DOI: 10.1016/j.ympev.2010.05.009.
138. Fawcett JA, Innan H. Neutral and non-neutral evolution of duplicated genes with gene conversion. Genes (Basel) 2011;2(1):191–209. DOI: 10.3390/genes2010191.
139. Gu Y, Xing S, He C. Genome-wide analysis indicates lineage-specific gene loss during papilionoideae evolution. Genome Biol Evol 2016;8(3):635–648. DOI: 10.1093/gbe/evw021.
140. Assis R, Conant G, Holland B, et al. Models for the retention of duplicate genes and their biological underpinnings. F1000Res 2023;12:1400. DOI: 10.12688/f1000research.141786.2.
141. Hu Y, Ewen-Campen B, Comjean A, et al. Paralog explorer: A resource for mining information about paralogs in common research organisms. Comput Struct Biotechnol J 2022;20:6570–6577. DOI: 10.1016/j.csbj.2022.11.041.
142. Singh NP, Krumlauf R. Diversification and functional evolution of HOX proteins. Front Cell Dev Biol 2022;10:798812. DOI: 10.3389/fcell.2022.798812.
143. Lynch M, Katju V. The altered evolutionary trajectories of gene duplicates. Trends Genet 2004;20(11):544–549. DOI: 10.1016/j.tig.2004.09.001.
144. Han MV, Demuth JP, McGrath CL, et al. Adaptive evolution of young gene duplicates in mammals. Genome Res 2009;19(5):859–867. DOI: 10.1101/gr.085951.108.
145. Kumar S, Stecher G, Suleski M, et al. MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. Mol Biol Evol 2024;41(12):msae263. DOI: 10.1093/molbev/msae263.
146. Jones DT, Taylor WR, Thornton JM. The rapid generation of mutation data matrices from protein sequences. Comput Appl Biosci 1992;8(3):275–282. DOI: 10.1093/bioinformatics/8.3.275.
147. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol Biol Evol 1987;4(4):406–425. DOI: 10.1093/oxfordjournals.molbev.a040454.
148. Harman JL, Reardon PN, Costello SM, et al. Evolution avoids a pathological stabilizing interaction in the immune protein S100A9. Proc Natl Acad Sci U S A 2022;119(41):e2208029119. DOI: 10.1073/pnas.2208029119.
149. Zhou H, Zhao C, Shao R, et al. The functions and regulatory pathways of S100A8/A9 and its receptors in cancers. Front Pharmacol 2023;14:1187741. DOI: 10.3389/fphar.2023.1187741.
150. Perez M, Hurm K, Liberles DA. Understanding functional evolution in orthologs and paralogs. J Mol Evol 2025;93(6):730–739. DOI: 10.1007/s00239-025-10274-4.
151. Albalat R, Canestro C. Evolution by gene loss. Nat Rev Genet 2016;17(7):379–391. DOI: 10.1038/nrg.2016.39.
152. Goh CS, Bogan AA, Joachimiak M, et al. Co-evolution of proteins with their interaction partners. J Mol Biol 2000;299(2):283–293. DOI: 10.1006/jmbi.2000.3732.
153. Leukert N, Vogl T, Strupat K, et al. Calcium-dependent tetramer formation of S100A8 and S100A9 is essential for biological activity. J Mol Biol 2006;359(4):961–972. DOI: 10.1016/j.jmb.2006.04.009.
154. Jauch-Speer SL, Herrera-Rivero M, Ludwig N, et al. C/EBPdelta-induced epigenetic changes control the dynamic gene transcription of S100a8 and S100a9. Elife 2022;11:e75594. DOI: 10.7554/eLife.75594.
155. Polakowska M, Steczkiewicz K, Szczepanowski RH, et al. Toward an understanding of the conformational plasticity of S100A8 and S100A9 $Ca^{(2+)}$-binding proteins. J Biol Chem 2023;299(4):102952. DOI: 10.1016/j.jbc.2023.102952.
156. Averill MM, Kerkhoff C, Bornfeldt KE. S100A8 and S100A9 in cardiovascular biology and disease. Arterioscler Thromb Vasc Biol 2012;32(2):223–229. DOI: 10.1161/ATVBAHA.111.236927.
157. Vogl T, Roth J, Sorg C, et al. Calcium-induced noncovalently linked tetramers of MRP8 and MRP14 detected by ultraviolet matrix-assisted laser desorption/ionization mass spectrometry. J Am Soc Mass Spectrom 1999;10(11):1124–1130. DOI: 10.1016/s1044-0305(99)00085-9.

158. Pathirana WGW, Chubb SP, Gillett MJ, et al. Faecal calprotectin. Clin Biochem Rev 2018;39(3):77–90. PMID: 30828114.
159. Azramezani Kopi T, Shahrokh S, Mirzaei S, et al. The role of serum calprotectin as a novel biomarker in inflammatory bowel diseases: A review study. Gastroenterol Hepatol Bed Bench 2019;12(3):183–189. PMID: 31528300.
160. Labaere D, Smismans A, Van Olmen A, et al. Comparison of six different calprotectin assays for the assessment of inflammatory bowel disease. United European Gastroenterol J 2014;2(1):30–37. DOI: 10.1177/2050640613518201.
161. Hamer HM, Mulder AHL, de Boer NK, et al. Impact of preanalytical factors on calprotectin concentration in stool: A multiassay comparison. J Appl Lab Med 2022;7(6):1401–1411. DOI: 10.1093/jalm/jfac057.
162. Wang W, Cao W, Zhang S, et al. The role of calprotectin in the diagnosis and treatment of inflammatory bowel disease. Int J Mol Sci 2025;26(5):1996. DOI: 10.3390/ijms26051996.
163. Albanna EA, Ahmed HS, Awad HA. Stool calprotectin in necrotizing enterocolitis. J Clin Neonatol 2014;3(1):16–19. DOI: 10.4103/2249-4847.128721.
164. Erol S, Tayman C, Korkut S, et al. A comparative study of serum and fecal calprotectin levels in necrotizing enterocolitis. J Pediatr (Rio J) 2025;101(5):101428. DOI: 10.1016/j.jped.2025.101428.
165. Campeotto F, Butel MJ, Kalach N, et al. High faecal calprotectin concentrations in newborn infants. Arch Dis Child Fetal Neonatal Ed 2004;89(4):F353–F355. DOI: 10.1136/adc.2002.022368.
166. Saviano A, Candelli M, Zanza C, et al. Gastrointestinal involvement in extra-digestive disease: Which is the role of fecal calprotectin? Medicina (Kaunas) 2022;58(10):1384. DOI: 10.3390/medicina58101384.
167. Maheshwari A. The phylogeny, ontogeny, and organ-specific differentiation of macrophages in the developing intestine. Newborn (Clarksville) 2022;1(4):340–355. DOI: 10.5005/jp-journals-11002-0044.
168. Jeong SJ. The role of fecal calprotectin in pediatric disease. Korean J Pediatr 2019;62(8):287–291. DOI: 10.3345/kjp.2019.00059.
169. Zeng J, Yu W, Gao X, et al. Establishing reference values for age-related fecal calprotectin in healthy children aged 0–4 years: A systematic review and meta-analysis. PeerJ 2025;13:e19572. DOI: 10.7717/peerj.19572.
170. Li F, Ma J, Geng S, et al. Fecal calprotectin concentrations in healthy children aged 1-18 months. PLoS One 2015;10(3):e0119574. DOI: 10.1371/journal.pone.0119574.
171. Jain SK, Baggerman EW, Mohankumar K, et al. Amniotic fluid-borne hepatocyte growth factor protects rat pups against experimental necrotizing enterocolitis. Am J Physiol Gastrointest Liver Physiol 2014;306(5):G361–G369. DOI: 10.1152/ajpgi.00272.2013.
172. Satrom KM, Ennis K, Sweis BM, et al. Neonatal hyperglycemia induces CXCL10/CXCR3 signaling and microglial activation and impairs long-term synaptogenesis in the hippocampus and alters behavior in rats. J Neuroinflammation 2018;15(1):82. DOI: 10.1186/s12974-018-1121-9.
173. Maheshwari A, Christensen RD, Calhoun DA. Immune neutropenia in the neonate. Adv Pediatr 2002;49:317–339. PMID: 12214777.
174. Maheshwari A. Role of platelets in neonatal necrotizing enterocolitis. Pediatr Res 2021;89(5):1087–1093. DOI: 10.1038/s41390-020-1038-8.
175. Namachivayam K, MohanKumar K, Garg L, et al. Neonatal mice with necrotizing enterocolitis-like injury develop thrombocytopenia despite increased megakaryopoiesis. Pediatr Res 2017;81(5):817–824. DOI: 10.1038/pr.2017.7.
176. Howarth C, Banerjee J, Eaton S, et al. Biomarkers of gut injury in neonates—where are we in predicting necrotising enterocolitis? Front Pediatr 2022;10:1048322. DOI: 10.3389/fped.2022.1048322.
177. Yoon JM, Park JY, Ko KO, et al. Fecal calprotectin concentration in neonatal necrotizing enterocolitis. Korean J Pediatr 2014;57(8):351–356. DOI: 10.3345/kjp.2014.57.8.351.
178. Ramineni P, Kamath SP, Manjrekar P, et al. Serum calprotectin as a marker of neonatal sepsis: A hospital-based cross-sectional diagnostic study. F1000Res 2023;12:626. DOI: 10.12688/f1000research.132099.2.
179. Mahjoub FE, Zahedi N, Ashjai B, et al. Role of fecal calprotectin in differentiating between Hirschsprung's disease and functional constipation. Korean J Gastroenterol 2013;62(5):288–291. DOI: 10.4166/kjg.2013.62.5.288.
180. Roychaudhuri S, Grewal G, Vijayashankar SS, et al. Necrotizing enterocolitis associated with congenital heart disease—A review article. Newborn (Clarksville) 2022;1(1):170–176. DOI: 10.5005/jp-journals-11002-0016.
181. Cremer A, Ku J, Amininejad L, et al. Variability of faecal calprotectin in inflammatory bowel disease patients: An observational case-control study. J Crohns Colitis 2019;13(11):1372–1379. DOI: 10.1093/ecco-jcc/jjz069.
182. Houston JFB, Morgan JE. Question 2: Can faecal calprotectin be used as an effective diagnostic aid for necrotising enterocolitis in neonates? Arch Dis Child 2015;100(10):1003–1006. DOI: 10.1136/archdischild-2015-309266.
183. Qu Y, Xu W, Han J, et al. Diagnostic value of fecal calprotectin in necrotizing enterocolitis: A meta-analysis. Early Hum Dev 2020;151:105170. DOI: 10.1016/j.earlhumdev.2020.105170.
184. Mumolo MG, Bertani L, Ceccarelli L, et al. From bench to bedside: Fecal calprotectin in inflammatory bowel diseases clinical setting. World J Gastroenterol 2018;24(33):3681–3694. DOI: 10.3748/wjg.v24.i33.3681.
185. Terrin G, Passariello A, Manguso F, et al. Serum calprotectin: An antimicrobial peptide as a new marker for the diagnosis of sepsis in very low birth weight newborns. Clin Dev Immunol 2011;2011:291085. DOI: 10.1155/2011/291085.
186. Park IH, Lee SH, Yu ST, et al. Serum procalcitonin as a diagnostic marker of neonatal sepsis. Korean J Pediatr 2014;57(10):451–456. DOI: 10.3345/kjp.2014.57.10.451.
187. Decembrino L, De Amici M, Pozzi M, et al. Serum calprotectin: A potential biomarker for neonatal sepsis. J Immunol Res 2015;2015:147973. DOI: 10.1155/2015/147973.
188. Whitehead SJ, Ford C, Gama RM, et al. Effect of faecal calprotectin assay variability on the management of inflammatory bowel disease and potential role of faecal S100A12. J Clin Pathol 2017;70(12):1049–1056. DOI: 10.1136/jclinpath-2017-204340.
189. Saviano A, Migneco A, Brigida M, et al. Serum calprotectin in the evaluation of gastrointestinal diseases: An ace up your sleeve? Medicina (Kaunas) 2024;60(5). DOI: 10.3390/medicina60050762.
190. D'Amico F, Rubin DT, Kotze PG, et al. International consensus on methodological issues in standardization of fecal calprotectin measurement in inflammatory bowel diseases. United European Gastroenterol J 2021;9(4):451–460. DOI: 10.1002/ueg2.12069.

CLINICAL TECHNIQUE

Clinical Imaging: Point-of-care Ultrasound for Endotracheal Tube Localization in Neonates: Why It Matters

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Received on: 25 October 2025; *Accepted on*: 05 December 2025; *Published on*: 08 April 2026

Abstract

Endotracheal tube (ETT) malposition is a frequently encountered difficulty in the management of critically ill infants in the neonatal intensive care units (NICUs). Chest radiography (CXR) still remains the gold standard for confirmation of correct positioning of the ETT, but it is limited by temporal delays, radiation exposure, and workflow inefficiencies. Point-of-care ultrasound (POCUS) offers a rapid, radiation-free, and reliable alternative for confirming the position of the ETT at the bedside. This review summarizes the clinical rationale, scanning technique, available evidence, and practical strategies for using POCUS for ETT localization in neonates.

Keywords: B-mode, Clinical rationale, Depth, Double trachea sign, Dynamic range, Endotracheal tube, Evidence, Focal zone, Frame rate, Gain, Hockey-stick probe, Infants, Linear high-frequency probe, Linear small-footprint or hockey-stick probe, Malposition, Microlinear probe, Newborn, Partial ossification, POCUS, Radiation exposure, Regression formula, Scanning, Sonography, Standardized protocols, Suprasternal notch, Tip-to-arch distance, Workflow inefficiencies.

Newborn (2026): 10.5005/jp-journals-11002-0141

Key Points

- Endotracheal tube (ETT) malposition is a frequently encountered difficulty in the management of critically ill infants in the neonatal intensive care units (NICUs).
- Point-of-care ultrasound (POCUS) offers a rapid, radiation-free, and reliable way for confirming ETT position at the bedside.
- In this article, we have summarized available information on the use of ultrasonography for ETT localization in neonates, including the scanning technique(s), supporting evidence, and practical strategies that we have found useful in the NICU.

Introduction

Accurate placement of the ETT is critical for effective ventilation and prevention of complications in neonates. Despite careful technique, malposition remains common, with studies reporting ETT misplacement rates up to 30% and umbilical venous catheter (UVC) malposition rates as high as 40% when confirmed radiographically.[1,2]

Chest radiography (CXR) continues to serve as the conventional gold standard for ETT verification; however, it introduces delays, exposes infants to ionizing radiation, and may disrupt workflow during critical stabilization. In contrast, POCUS enables real-time confirmation of ETT position within minutes at the bedside, facilitating prompt correction and minimizing the risk of hypoventilation, atelectasis, or lung injury. This article reviews the rationale, technical aspects, and implementation of POCUS for ETT localization in neonatal intensive care practice.

Clinical Importance of ETT Position

Malposition of the ETT can have serious consequences. A high ETT position risks accidental extubation and CO_2 retention, while a low-lying tube can cause unilateral ventilation, atelectasis, or barotrauma.[3] Furthermore, ETT depth may change dynamically with head and neck movement—flexion or extension can shift the tip position by up to 2 cm.[4] Relying solely on delayed radiographic confirmation may prolong periods of hypoxia or ineffective ventilation. Early bedside confirmation using ultrasound, therefore, provides a clinically significant advantage in preventing avoidable morbidity (Fig. 1).

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How to cite this article: Kaushal M, Balla KC. Clinical Imaging: Point-of-care Ultrasound for Endotracheal Tube Localization in Neonates: Why It Matters. Newborn 2026;5(1):58–60.

Source of support: Nil

Conflict of interest: None

Our ETT-POCUS Algorithm

Clinical Technique for Placement and Monitoring

- Intubate and maintain a neutral head position.
- Place probe horizontally over suprasternal notch (marker right).
- Confirm single tracheal structure and absence of the double trachea sign.
- Rotate the probe perpendicular to identify the aortic arch and ETT tip.
- Measure tip-to-arch distance.
- Adjust ETT by 1–2 mm and re-scan.
- Document measurements.
- Obtain CXR if discrepancy or policy requires.

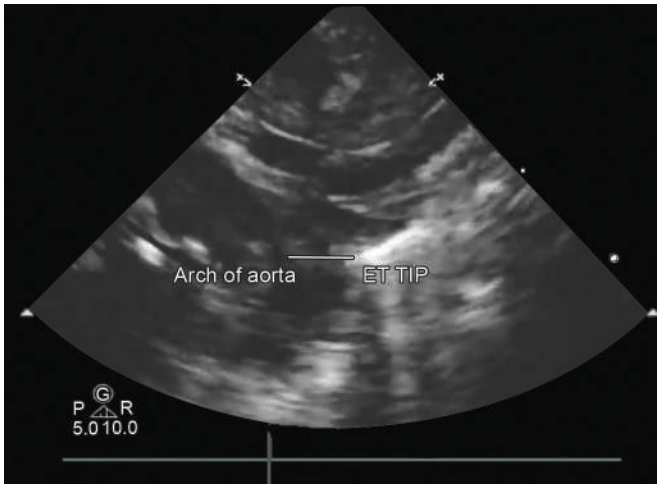

Fig. 1: Sonography shows a hyperlucent ETT in the trachea. A distance of 5–10 mm (marked) between the tip of the ETT and the aortic arch is consistent with optimal placement of the tube

Probe Selection by Gestational Age

Gestational group	*Recommended probe*	*Frequency range*	*Rationale*
Term and late preterm (>34 weeks)	Linear high-frequency probe	8–12 MHz	Optimal penetration and detail of trachea/ETT tip
Preterm (28–32 weeks)	Linear small-footprint or hockey-stick probe	10–15 MHz	Better surface contact, improved near-field resolution
ELBW (<28 weeks)	Microlinear or hockey-stick probe	12–18 MHz	Excellent resolution for superficial structures

ELBW, extreme low birth weight

Machine Settings

Mode: B-mode
Depth: 1.5–3 cm
Focal zone: At the tracheal level
Gain: Moderate to low
Dynamic range: 50–70 dB
Frame rate: >30 fps

Optimal Tip-to-arch Distances

Infant group	*Ideal tip-to-arch distance (mm)*	*Reference*
Term (>1,500 gm)	6–9	Şahin et al., 2024[5]
VLBW (<1,500 gm)	5–8	Singh et al., 2019[6]
ELBW (<1,000 gm)	≈7–9 (Regression formula)	Levkovitz et al., 2023[7]

Evidence Supporting ETT-POCUS

Recent studies have validated ultrasound-based measurements against radiography. Levkovitz et al. demonstrated a strong correlation ($r = 0.83$) between ultrasound-measured ETT–AA distance and radiographic tip position.[7] Şahin et al. reported 92% concordance between ultrasound-confirmed ETT positions and CXR-confirmed placement at tracheal levels T1–T3.[5] Meta-analyses further support high specificity and workflow efficiency with POCUS compared to traditional CXR.[8,9]

Pitfalls and Pearls

- Poor visualization in ELBW infants: Use higher-frequency probes (10–15 MHz).
- Partial ossification: Slightly reduces the acoustic window but remains interpretable.
- Gentle probe pressure: Prevents tracheal compression or vagal response.
- Anatomic variations: Confirm in multiple planes.
- Short tracheal length: Even minor tube adjustments can cause malposition.
- Head movement: Stabilize during and after scanning.
- Reintubation: Always reconfirm ETT location.

Influence of Polymers Used to Prepare ETTs on Ultrasound Visibility

Neonatal ETTs are commonly made of polyvinyl chloride (PVC) or silicone-based soft-flex materials. In our experience, PVC tubes show higher echogenicity and clearer visualization than those composed of silicone (soft-flex tubes); the acoustic properties of the latter are closer to those of human soft tissues.[6,10–12]

Implementation in NICU Practice

Successful integration of ETT-POCUS requires structured training, workflow checklists, image archiving for quality assurance, and policy updates to designate ultrasound as a first-line verification tool. Early adopter NICUs report improved efficiency, reduced repeat CXRs, and greater clinician confidence.

Conclusion

Point-of-care ultrasound provides a rapid, accurate, and radiation-free method for confirming ETT position in neonates. Currently available evidence supports its reliability and shows a strong correlation with radiographic confirmation. Standardized protocols and regular competency assessments can help establish POCUS as the preferred first-line tool for airway verification in NICUs.

References

1. Singh Y, Tissot C, Fraga MV, et al. International evidence-based guidelines on Point-of-care ultrasound (POCUS) for neonates and children. Lancet Child Adolesc Health 2020;4(12):853–864. DOI: 10.1186/s13054-020-2787-9.
2. Ades A, Sable C, Cummings S, et al. Echocardiographic evaluation of umbilical venous catheters in neonates. J Perinatol 2017;37(4):406–410. DOI: 10.1038/sj.jp.7210851.
3. Dennington D, Vali S, Sinha A. Ultrasound confirmation of endotracheal tube position in neonates. NeoReviews 2012;13(3):e185–e192. DOI: 10.1159/000338585.
4. Najib K, Pishdad P, Gharagozlou M, et al. Ultrasonographic confirmation of endotracheal tube tip-to-carina distance in infants. Indian Pediatr 2016;53(10):887–891. PMID: 27771669.
5. Sahin O, Tasar S, Colak D, et al. Point-of-Care ultrasound for the tip of the endotracheal tube: A neonatologi st perspective. Am J Perinatol 2024;41(S01):e2886–e2892. DOI: 10.1055/a-2181-7354.

6. Singh P, Thakur A, Garg P, et al. Normative data of optimally placed endotracheal tube by point-of-care ultrasound in neonates. Indian Pediatr 2019;56:374–380. PMID: 31102379.
7. Levkovitz O, Levy Y, Yitzhaki M, et al. Ultrasound assessment of endotracheal tube depth in neonates: A prospective feasibility study. J Ultrasound Med 2023;109(1):94–99. DOI: 10.1136/archdischild-2023-325855.
8. Gürsoy BK, Çelik A, Öztürk H, et al. Ultrasonographic evaluation of endotracheal tube position in newborns with congenital heart disease. Cardiol Young 2023;33(3):305–312. DOI: 10.1017/S1047951122003420.
9. Dincer E, Topçuoğlu S, Karatekin G. Ultrasonography for determining endotracheal tube tip: Review and practicality. J Ultrasound Med 2023;42(2):437–441. DOI: 10.1002/jum.16067.
10. Subramani S, Parameswaran N, Ananthkrishnan R, et al. Assessment of the endotracheal tube tip position by portable ultrasound—An observational study. Indian J Crit Care Med 2022;26(11):1243–1248. DOI: 10.5005/jp-journals-10071-24355.
11. Chellen S, Miall L. In neonates who have been successfully intubated can ultrasound be used to accurately confi rm endotracheal tube tip position? Arch Dis Child 2022;107(3):305–308. DOI: 10.1136/archdischild-2021-323146.
12. Alsabri M, Abady E, Hasan MT, et al. Diagnostic accuracy of real-time point-of-care tracheal ultrasound to confirm ETT placement in neonates: Systematic review & meta-analysis. J Perinatol 2025. DOI: 10.1038/s41372-025-02461-4.

CASE REPORT

Radiology Case Reports: Midgut Malrotation without Volvulus

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Received on: 02 February 2026; *Accepted on*: 05 March 2026; *Published on*: 08 April 2026

Abstract

Intestinal malrotation is a congenital condition in which the intestines become fixed in an abnormal position during fetal development and do not rotate further. This condition presents most frequently during infancy with symptoms such as bilious vomiting and abdominal distension, although some individuals may remain asymptomatic or develop chronic abdominal pain, nausea, or vomiting later in childhood or adolescence. The diagnosis is typically made using imaging studies, and treatment usually involves surgical correction to prevent serious complications. The pathophysiology of malrotation is not well known; there is an error in embryologic development of the midgut between weeks 5–10 of gestation, when the intestine normally herniates, undergoes a 270-degree counterclockwise rotation, and then returns to the abdominal cavity. Mutations or altered expression of genes involved in laterality and gut morphogenesis, such as *forkhead box F1 (FOXF1)*, paired-like homeodomain 2 (PITX2), and components of the nodal growth differentiation factor (NODAL) signaling pathway, have been implicated. Here, we present plain and contrast-enhanced radiological images from an infant who presented with signs of intestinal obstruction. The management of intestinal malrotation primarily involves supportive management and surgical intervention to prevent life-threatening complications such as midgut volvulus. The standard treatment is the Ladd procedure, which includes detorsion of the bowel if volvulus is present, division of obstructing Ladd bands, widening of the mesenteric base to reduce the risk of future twisting, and repositioning of the intestines in a nonrotated configuration.

Keywords: Asplenia, Case report, Copy number variants, Development, *Forkhead box F1*, Intestinal herniation, Intestinal rotation, Intestine, Ladd band, Ladd procedure, Meckel's diverticulum.

Newborn (2026): 10.5005/jp-journals-11002-0148

Key Points

- Midgut malrotation is a congenital condition in which the intestines become fixed in an abnormal position during fetal development and do not rotate further.
- The pathophysiology of malrotation is not well known. There is an error in embryologic development of the midgut between weeks 5–10 of gestation.
- Mutations or altered expression of genes involved in laterality and gut morphogenesis, such as *forkhead box F1 (FOXF1)*, paired-like homeodomain 2 (PITX2), and components of the nodal growth differentiation factor (NODAL) signaling pathway, have been implicated, particularly in patients with heterotaxy or other congenital anomalies.
- Management of intestinal malrotation primarily involves supportive management and surgical intervention to prevent life-threatening complications such as midgut volvulus. The standard treatment is the Ladd procedure, which includes detorsion of the bowel if volvulus is present and division of the obstructing Ladd bands.

Intestinal malrotation is a congenital condition in which the intestines become fixed in an abnormal position during fetal development and do not rotate further.[1] Consequently, the bowel is seen abnormally positioned in the abdomen and may be inadequately anchored, increasing the risk of intestinal obstruction or twisting of the bowel, known as volvulus.[2] This condition presents most frequently during infancy with symptoms such as bilious vomiting and abdominal distension, although some individuals may remain asymptomatic or develop chronic abdominal pain, nausea, or vomiting later in childhood or adolescence. Diagnosis is typically made using imaging studies, and treatment usually involves surgical correction to prevent serious complications.

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How to cite this article: Rao AG. Radiology Case Reports: Midgut Malrotation without Volvulus. Newborn 2026;5(1):61–64.

Source of support: Nil

Conflict of interest: None

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

Case Description

Here, we present plain and contrast-enhanced radiological images from a 1-week-old male infant who presented with bilious vomiting for which an upper gastrointestinal (UGI) series study was done (Figs 1 to 5). The findings were consistent with a midgut malrotation without volvulus:

Figure 1: Frontal supine abdomen radiograph showing a gas-distended stomach without bowel obstruction.

Figures 2 and 3: Fluoroscopic image in the right lateral decubitus position (Fig. 2) and supine frontal view (Fig. 3) following

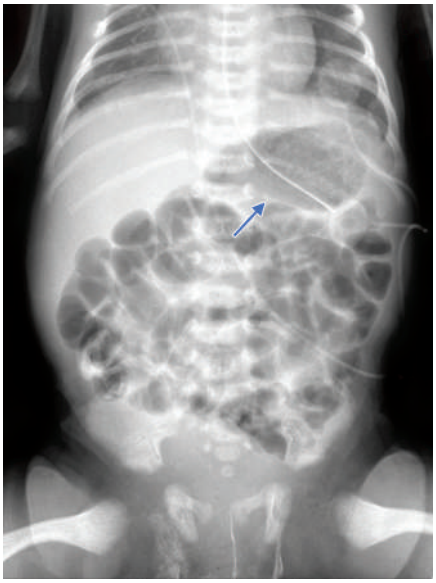

Fig. 1: Scout supine frontal abdomen radiograph showing a distended stomach (arrow) with a nonspecific nonobstructive bowel gas pattern

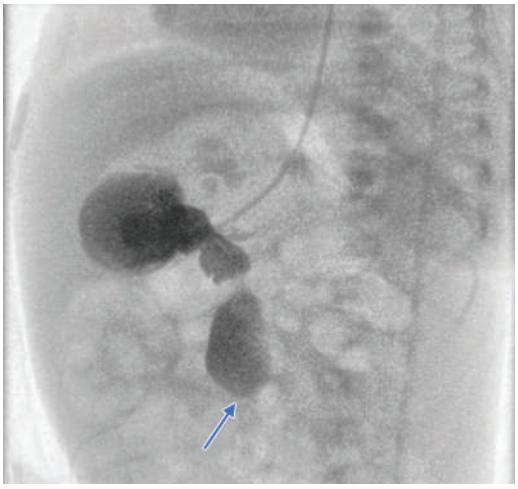

Fig. 2: Right lateral decubitus fluoroscopic image of abdomen after water-soluble contrast injection into the stomach showing dilated second portion of the duodenum (arrow)

water soluble contrast injection into the stomach through the nasogastric tube, showing dilated stomach and dilated first and second portions of the duodenum with narrowing at the proximal third portion of the duodenum. The second portion also has a retroperitoneal course.

Figures 4 and 5: Fluoroscopic right lateral decubitus and supine frontal views showing progression of contrast to the distal portions of the duodenum and proximal jejunum with the focal transition/stenosis in the proximal third portion. This was reported as duodenal obstruction/stenosis, and the duodenojejunal junction was felt to be normal on this study.

At surgery, duodenal obstruction at this site was seen due to Ladd's bands, and the patient also had midgut malrotation. Ladd's procedure was performed, and the patient was discharged following an uneventful postsurgical recovery. This case highlights

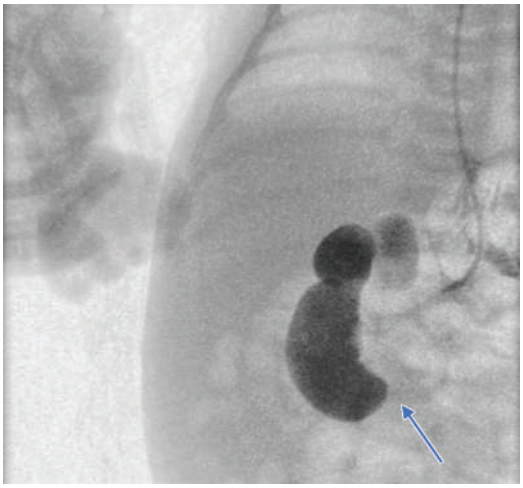

Fig. 3: Supine frontal fluoroscopic image showing dilated duodenum with tapering in its proximal third portion (arrow)

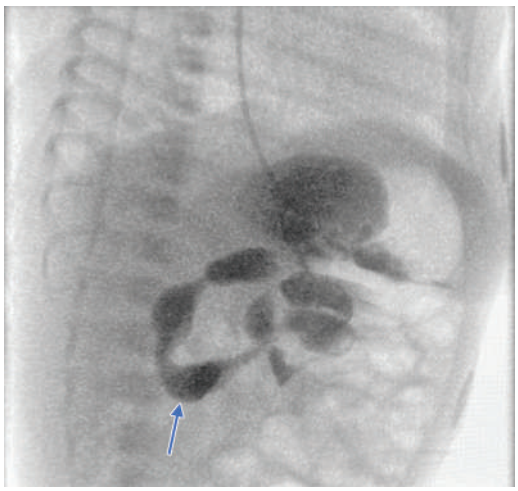

Fig. 4: Left lateral decubitus fluoroscopic image showing the appearance of the retroperitoneal course of the duodenum (arrow) with contrast flowing into proximal jejunum, excluding complete obstruction

that an UGI series study is not 100% sensitive or specific for the diagnosis of midgut malrotation and midgut volvulus, even though it is done as the first line of imaging evaluation for these entities. False positive and false negative results can therefore happen.

The pathophysiology of malrotation is not well known. There is an error in embryologic development of the midgut between weeks 5–10 of gestation, when the intestine normally herniates, rotates 270 degrees counterclockwise around the superior mesenteric artery, and then returns to the abdominal cavity.[3] In malrotation, this rotational process is incomplete or abnormal, leading to improper positioning of the duodenum and cecum and inadequate fixation of the mesentery.[4] The resulting narrow mesenteric base predisposes the bowel to twisting (midgut volvulus), while abnormal peritoneal bands (Ladd bands) may form and compress the duodenum, causing obstruction.[1]

Malrotation is usually sporadic, though familial cases and associations with genetic syndromes suggest a heritable

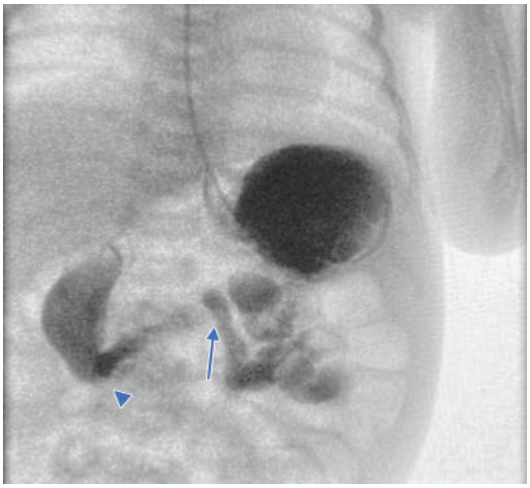

Fig. 5: Supine frontal fluoroscopic image of the abdomen showing a transition in the lumen of the duodenum in its proximal third portion (arrowhead), indicating obstruction and appearance of normal position of the duodenojejunal junction (arrow) to the left of the spine and almost at the same level or in retrospect, at a lower level than the first portion of the duodenum. This can be seen when the stomach is distended

component.[5] The genetic pathogenesis of intestinal malrotation is not fully understood. There are anatomic disruptions in embryonic left–right patterning, midgut rotation, and mesenteric fixation during early development.[5,6] Mutations or altered expression of genes involved in laterality and gut morphogenesis, such as *FOXF1*, paired-like homeodomain 2, and components of the NODAL signaling pathway, have been implicated, particularly in patients with heterotaxy or other congenital anomalies.[6–9] Copy number variants have also been noted.[10] These genetic disturbances can possibly interfere with normal midgut rotation around the superior mesenteric artery, leading to abnormal intestinal positioning and fixation that might increase the risk of obstruction and volvulus.[6]

Intestinal malrotation is frequently associated with other congenital anomalies, particularly those involving abnormal embryologic development and left–right axis formation. It commonly occurs in association with congenital diaphragmatic hernia, abdominal wall defects such as omphalocele and gastroschisis, and cardiac anomalies, especially in patients with heterotaxy syndromes.[11–15] Malrotation may also be seen alongside intestinal atresias, Meckel's diverticulum, and anomalies of the spleen, including asplenia or polysplenia.[16–18] These associated conditions reflect a broader disturbance in embryonic development and often influence the timing of diagnosis, clinical presentation, and overall management of intestinal malrotation.[1]

Management of intestinal malrotation primarily involves supportive management and surgical intervention to prevent life-threatening complications such as midgut volvulus.[4,19] The standard treatment is the Ladd procedure, which includes detorsion of the bowel if volvulus is present, division of obstructing Ladd bands, widening of the mesenteric base to reduce the risk of future twisting, and repositioning of the intestines in a nonrotated configuration; an appendectomy is often performed to avoid diagnostic confusion later.[20–22] Surgery is recommended even in many asymptomatic patients due to the unpredictable risk of volvulus.[23]

References

1. Holt AC, Anand S, Nada H, et al. Midgut Volvulus. StatPearls. 2022 2025. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441962/. Accessed on: 01 February 2026.
2. Ferrero L, Ahmed YB, Philippe P, et al. Intestinal malrotation and volvulus in neonates: Laparoscopy versus open laparotomy. J Laparoendosc Adv Surg Tech A 2017;27(3):318–321. DOI: 10.1089/lap.2015.0544.
3. Galaz FP, Rico KM, Tristan FAP, et al. Midgut volvulus caused by intestinal malrotation: A rare cause of acute abdomen in adults. Case report. Int J Surg Case Rep 2020;73:355–359. DOI: 10.1016/j.ijscr.2020.07.051.
4. Svetanoff WJ, Srivatsa S, Diefenbach K, et al. Diagnosis and management of intestinal rotational abnormalities with or without volvulus in the pediatric population. Semin Pediatr Surg 2022;31(1):151141. DOI: 10.1016/j.sempedsurg.2022.151141.
5. Grzymkowski J, Wyatt B, Nascone-Yoder N. The twists and turns of left-right asymmetric gut morphogenesis. Development 2020;147(19). DOI: 10.1242/dev.187583.
6. Martin V, Shaw-Smith C. Review of genetic factors in intestinal malrotation. Pediatr Surg Int 2010;26(8):769–781. DOI: 10.1007/s00383-010-2622-5.
7. Miranda J, Rocha G, Soares P, et al. A novel mutation in *FOXF1* gene associated with alveolar capillary dysplasia with misalignment of pulmonary veins, intestinal malrotation and annular pancreas. Neonatology 2013;103(4):241–245. DOI: 10.1159/000346062.
8. Sanketi BD, Zuela-Sopilniak N, Bundschuh E, et al. *Pitx2* patterns an accelerator-brake mechanical feedback through latent TGFbeta to rotate the gut. Science 2022;377(6613):eabl3921. DOI: 10.1126/science.abl3921.
9. Olutoye OO 2nd, Hammond JD 2nd, Gilley J, et al. Fetal malrotation with midgut volvulus: Prenatal diagnosis and planning. J Pediatr Surg Case Rep 2023;93. DOI: 10.1016/j.epsc.2023.102654.
10. Karlslätt KS, Pettersson M, Jäntti N, et al. Rare copy number variants contribute pathogenic alleles in patients with intestinal malrotation. Mol Genet Genomic Med 2019;7(3):e549. DOI: 10.1002/mgg3.549.
11. Abdelhafeez AH, Schultz JA, Ertl A, et al. The risk of volvulus in abdominal wall defects. J Pediatr Surg 2015;50(4):570–572. DOI: 10.1016/j.jpedsurg.2014.12.017.
12. Fawley JA, Abdelhafeez AH, Schultz JA, et al. The risk of midgut volvulus in patients with abdominal wall defects: A multi-institutional study. J Pediatr Surg 2017;52(1):26–29. DOI: 10.1016/j.jpedsurg.2016.10.014.
13. Mahalik SK, Khanna S, Menon P. Malrotation and volvulus associated with heterotaxy syndrome. J Indian Assoc Pediatr Surg 2012;17(3):138–140. DOI: 10.4103/0971-9261.98138.
14. Wen CC, Kuo TC, Lee HC, et al. Coexisting gastrointestinal and hepatobiliary tract anomalies in omphalocele and gastroschisis: A twenty-year experience in a single tertiary medical center. Pediatr Neonatol 2022;63(5):468–473. DOI: 10.1016/j.pedneo.2022.03.009.
15. Kouwenberg M, Severijnen RS, Kapusta L. Congenital cardiovascular defects in children with intestinal malrotation. Pediatr Surg Int 2008;24(3):257–263. DOI: 10.1007/s00383-007-2086-4.
16. Nakada K, Kawaguchi F, Wakisaka M, et al. Digestive tract disorders associated with asplenia/polysplenia syndrome. J Pediatr Surg 1997;32(1):91–94. DOI: 10.1016/s0022-3468(97)90103-2.
17. Ditchfield MR, Hutson JM. Intestinal rotational abnormalities in polysplenia and asplenia syndromes. Pediatr Radiol 1998;28(5):303–306. DOI: 10.1007/s002470050358.
18. Vasudayan SR, Loo GH, Sellapan M, et al. Small bowel volvulus secondary to Meckel's diverticulum with meso-diverticular band: A case report and literature review. Cureus 2025;17(5):e83817. DOI: 10.7759/cureus.83817.
19. Bostancı SA, Öztorun CI, Erten EE, et al. Clinical management of intestinal malrotation in different age groups. Pediatr Surg Int 2024;40(1):204. DOI: 10.1007/s00383-024-05796-9.

20. Nakayama DK. William Ladd's procedure for malrotation and volvulus: Convincing the world. J Pediatr Surg 2025;60(11):162645. DOI: 10.1016/j.jpedsurg.2025.162645.
21. Al Smady MN, Hendi SB, AlJeboury S, et al. Appendectomy as part of Ladd's procedure: A systematic review and survey analysis. Pediatr Surg Int 2023;39(1):164. DOI: 10.1007/s00383-023-05437-7.
22. Catania VD, Lauriti G, Pierro A, et al. Open versus laparoscopic approach for intestinal malrotation in infants and children: A systematic review and meta-analysis. Pediatr Surg Int 2016;32(12):1157–1164. DOI: 10.1007/s00383-016-3974-2.
23. Holzemer J, Patel K, Ruiz O, et al. Surgical management of congenital malrotation presenting in adulthood. BMJ Case Reports 2023;16:e253848. DOI: 10.1136/bcr-2022-253848.