



MODERN JOURNAL OF HEALTH AND APPLIED SCIENCES

INTERNATIONAL SCIENTIFIC JOURNAL
ISSUED BY
MODERN UNIVERSITY COLLEGE

VOLUME 3. ISSUE 2

RAMALLAH - PALESTINE

2026



Modern Journal of Health and Applied Sciences (MJHAS)

Editor-in-chief

Dr. Rebhi Bsharat, Surgical Nursing, Modern University College, Palestine.

Managing editor

Dr. Oday Khamaysa, Materials Chemistry, Modern University College, Palestine.

Editorial board members

Dr. Lgaz Hassane, Fundamental and Applied Chemistry, Hanyang University, South Korea.

Dr. Ahmed Nasr Alabssawy, Fish Biology and Ecology and Aquaculture, Al-Azhar University, Egypt.

Prof. Merzoug Benahmed, Organic Chemistry, University of Larbi Ben M'hidi, Algeria.

Dr. Mohamed Ahmed Ali Hussein, Inorganic Chemistry, Badr University, Egypt.

Dr. Moh'd Rasoul Ahmad Al-Hadidi, Electronics and Computer Engineering, Al-Balqa Applied University, Jordan.

Dr. Samer Khalaf, Biotechnology & Bioecosystem, Modern University College, Palestine.

Prof. Luma Ibrahim Khalil Ahmed Al-Allaf, Anatomy and Histology, University of Mosul, Iraq.

Dr. Ghassan Ali Ameen Abu-Hijleh, Anatomy Medicine, An-Najah National University, Palestine.

Dr. Ilhem Selatnia, Material Chemistry, University of Larbi Ben M'hidi, Algeria.

Prof. Md Zuki bin Abu Bakar Zakaria, Veterinary Medicine Universiti Putra Malaysia, Malaysia.

Dr. Ahmed M. Seyam, Civil Engineering, Budapest University of Technology and Economics, Budapest.

Dr. Amjad S. Altaweel, Chemical Engineering, Modern University College, Palestine.

Dr. Andaleeb Mohammed Ahmad Abu Kamel, Community Health Nursing-Caregiver Health, Al-Zaytoonah University, Jordan.

Dr. Intima A. Alrimawi, Nursing Studies, Georgetown University, USA.

Dr. Ahmed Hanani, Medicine and Health Sciences, An-Najah National University, Palestine.

Dr. Ali Ay, Institute of Health Sciences/Nursing, Bingol University, Turkey.

Advisory Board Members

Prof. Loutfy H. Madkour, Physical chemistry, Nanoscience and Nanotechnology, Tanta University, Egypt.

Dr. Saffanah Khuder Mahmood, Veterinary Medicine/ Anatomy, University of Mosul, Iraq.

Dr. Zunita Zakaria, Molecular Biology, Universiti Putra Malaysia, Malaysia.

Dr. Tariq A. Al-Azab, Mechanical Engineering-Technical Sciences, Al-Balqa' Applied University, Jordan.

Dr. Mousa Mohammad Ahmad Imran, Physics, Al-Balqa Applied University, Jordan.

Dr. Ali A. Taani, Astrophysics and Space Science, Al-Balqa Applied University, Jordan.

Dr. Debab Houria, Applied Chemistry, University de Sétif, Algeria.

Dr. Mohammad Al-Talahma, Clinical Human Anatomy, Palestine Polytechnic University, Palestine.

Dr. Mustafa Shouli, Nursing Science - Community Health Nursing, Modern University College, Palestine.

Dr. Mohammed Hawash, Pharmaceutical Chemistry, An-Najah National University, Palestine.

Dr. Ahmad Khaled Taleb Darwazah, Cardiac Surgery, Menoufia University, Egypt.

Journal Coordinator

Mahmood Namora, Modern University College, Palestine.

Proofreader

Tala Al-Hilu, Modern University College, Palestine.

Technical Support

Giants Tech, Palestine.

Table of contents

<i>Title</i>	<i>Page</i>
Static Stretch on Calf Muscle Spasticity in Cerebral Palsy Children	1-08
Barriers to Injury Prevention in University-Level Contact Sports	09-16
Harnessing Bioinformatics to Decode Drug Resistance and Guide Vaccine Design in Mycobacterium tuberculosis	17-30
Evaluating Neonatal CPR Diagnostic Accuracy	31-41
Cytokine-Mediated Immune Responses in Mycobacterium tuberculosis Infections: Mechanisms, Regulation, and Therapeutic Implications	42-60
Synthetic Morphogen Gradient Engineering in Human Organoids: Toward Programmable Tissue Patterning for Next-Generation Regenerative Medicine	61-90
Radiologic Evaluation of the Upper Airway Before Anaesthesia	91-109
A Conceptual Framework for Relativistic Neuroscience: Temporal Layer Decoupling During Extreme Time Dilation	110-124



Static Stretch on Calf Muscle Spasticity in Cerebral Palsy Children

Fatima Alawad ¹ , Hassan Abdelnour ^{2,3*} , and Wisal Ahmed ^{4,5} 

¹ Faculty of Physiotherapy, Al Neelain University, Khartoum, Sudan.

² Department of Rehabilitation Sciences, College of Medical Sciences, Northern Border University, Arar, KSA.

³ Department of Physiotherapy, School of Health Sciences, Ahfad University for Women, Omdurman, Sudan.

⁴ Department of Pediatrics, Faculty of Medicine, Al Neelain University, Khartoum, Sudan.

⁵ Pediatric Specialists, Department of Pediatrics, Sultan Qaboos Hospital, Dhofar, Oman.

ARTICLE INFO

Received: 03rd March 2026

Received in revised form: 21st May 2026

Accepted: 29th May 2026

Published: 15th September 2026

*Corresponding Author:

Hassan Abdelnour

E-mail :

hasphysio@gmail.com

Citation : Alawad , F, Abdelnour, H, & Ahmed, W. (2026). Static Stretch on Calf Muscle Spasticity in Cerebral Palsy Children. *Modern Journal of Health and Applied Sciences*, 3(2), 01-08.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Cerebral palsy (CP) encompasses a group of developmental disorders affecting movement and posture, leading to activity limitations. These conditions stem from non-progressive disturbances in the brain that occur during fetal or infant development. Spastic cerebral palsy is the most common, affecting about 70% to 80% of children with the condition. Functional and clinical assessments were performed pre- and post-intervention using the Modified Ashworth Scale (MAS) to evaluate calf muscle spasticity and goniometry to measure the Range of Motion (ROM) for passive ankle dorsiflexion. Structured questionnaires used to assess socioeconomic and functional status. The intervention consisted of static muscle stretching for the calf muscles to reduce spasticity and increase ankle range of motion. Data were analyzed using SPSS version 21. All ethical considerations were maintained.

The study involved 26 participants, primarily characterized by quadriplegic cerebral palsy (CP) (66%, n = 17) and high levels of motor disability, with 53.8% (n=14) classified at Gross Motor Function Classification System (GMFCS) Level 5. Clinical evaluations focusing on spasticity using the Modified Ashworth Scale (MAS) showed no statistically significant reduction following the intervention; instead, mean scores increased from 2.0 to 2.5 on the right side (p = 0.916) and from 1.9 to 2.3 on the left side (p = 0.437). The treatment produced significant improvements in joint flexibility. Paired sample t-tests for passive ankle dorsiflexion range of motion (ROM) revealed a statistically significant increase on both the right side (12° to 15°; p = 0.001) and the left side (12° to 15°; p = 0.004). Despite no positive change in calf muscle spasticity, the intervention significantly improved ankle dorsiflexion ROM. This helps high-dependency patients reach the mobility levels needed for functional gait.

Keywords: Static stretch, Cerebral Palsy (CP), Spasticity, Calf muscle.

1. Introduction

Cerebral palsy (CP) refers to a set of lifelong neurological disorders that emerge during infancy and result from abnormal brain development or damage. These non-progressive conditions lead to enduring challenges with movement, posture, and coordination. The incidence rate is around 3.4 per 1,000 live births, making it the most common motor impairment, which can also affect cognitive and intellectual abilities (Almalty et al., 2025).

Spasticity refers to a condition where muscles become stiff and rigid, accompanied by overactive reflexes. This results from damage to the brain or spinal cord and often leads to involuntary muscle spasms, pain, and limited mobility (National Institute of Neurological Disorders and Stroke, 2023). Spasticity is frequently occurring in individuals affected by CP. Spasticity impacts over 12 million people globally, with prevalence rates differing widely depending on the associated neurological condition. It is most commonly observed in individuals with CP, affecting between 70% and 90% of patients (Barnes & Johnson, 2008).

Stretching is a physical activity focused on intentionally flexing and extending muscles and tendons to enhance elasticity, flexibility, and the range of motion in joints. Stretching serves as an essential, evidence-supported, and non-invasive approach to managing spasticity in individuals with CP. It helps decrease muscle stiffness, enhances range of motion (ROM), and minimizes the risk of contractures. Stretching interventions for spastic CP produce mixed outcomes, with research indicating an average improvement of around 10° in maximum ankle dorsiflexion after stretching (Kruse et al., 2022). Additionally, acute stretching may result in a 14.6% elongation of the muscle-tendon unit.

Calf muscle spasticity, commonly known as equinus gait, is highly prevalent in cases of spastic CP, impacting a significant proportion of children with diplegia or hemiplegia (Graham et al., 2016a). Among the 60–80% of CP cases classified as spastic, lower limb spasticity is a frequent concern. Research shows particularly high rates of rigid equinus, with some studies reporting its occurrence in over 90% of the populations examined (Horsch et al., 2021).

Sudan, known officially as the Republic of Sudan, is a nation situated in Northeast Africa (Horsch et al., 2021). Spasticity represents the most common type of CP in Sudan, encompassing a substantial majority of cases. Research consistently identifies spastic quadriplegic CP as the predominant subtype, with reported frequencies ranging from 43.5% to 86.6%. Among Sudanese children, spastic forms account for over 69% of cases, often attributed to perinatal factors such as asphyxia (Satti et al., 2026).

Ustratuna Sudanese Association for Disabled Children (USADC) is a non-governmental organization (NGO) founded in 1999 (Abdullahi et al., 2013). It has maintained a long-term partnership with the Italian NGO, Organismo di Volontariato per la Cooperazione Internazionale (OVCI). The organization focuses on providing rehabilitation services for children with disabilities, including spastic CP conditions (Abdelnour, 2020). Therefore, this study aimed to evaluate the effect of stretching on the spastic calf muscle in CP children at USADC.

2. Methodology

This study employed a quasi-experimental, pre- and post-interventional design focusing on a single cohort of children with spastic-type CP. The research was conducted at the USADC, located on Arda Street in Omdurman, Khartoum, Sudan. The center serves approximately 35 cases monthly, primarily children with neurological and musculoskeletal disorders. In collaboration with Ahfad University for Women (AUW) and OVCI, the center provides comprehensive, cost-free physiotherapy and rehabilitation services to the community.

The target population consisted of children diagnosed with CP, aged 2 to 10 years, presenting with calf muscle spasticity of Grade 3 or less on the Modified Ashworth Scale (MAS). For the sampling technique, total coverage sampling was used. For Sample Size, 26 children included. Due to the specific clinical population, a total coverage sampling technique was used, enrolling all 26 eligible children who met the inclusion criteria during the study period. No a priori power analysis was performed, and this sample size should be considered exploratory, particularly for detecting changes in spasticity.

Data collection took place between March 8, 2022, and September 27, 2022. Data were collected through structured questionnaires to capture participants' socioeconomic status and functional levels. Clinical assessments were conducted twice (pre-intervention and post-intervention) using the following tools. First, the MAS score was used to measure the grade of spasticity in the calf muscles of both the right and left lower limbs. Second, the goniometry was used to measure the range of motion (ROM) for passive ankle dorsiflexion.

The intervention consisted of a structured static stretching protocol targeting the spastic calf muscles. Each participant received manual static stretching for 3 sets of 30 repetitions, with each stretch held for 5 seconds. Sessions were conducted 3 times a week over a duration of 3 months (12 weeks).

Two independent physiotherapists with BSc degrees helped in data collection. The two physiotherapists underwent a standardized four-day training program prior to the intervention. The training focused on calf muscle static stretching and the assessment of ROM and MAS.

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 21. Descriptive statistics summarized the demographic and functional data. A paired t-test was utilized to compare the pre-test and post-test means to determine the intervention's efficacy. The threshold for statistical significance was set at $p < 0.05$.

Ethical approval was granted by the Institutional Review Board of Al Neelain University (NU). Administrative authorization was obtained from the USADC management. Informed consent was obtained from each participant's caregiver through a signed form. This document clearly outlined the study's objectives, procedures, and duration while explicitly stating the right to voluntary participation and withdrawal at any stage without penalty.

3. Results

This study included 26 pediatric patients with cerebral palsy, aged 2 to 9 years, along with their primary caregivers. Demographic data showed a higher prevalence of females (57.7%) compared to males (42.3%).

Table 1, illustrates the distribution of motor disability levels among the 26 study participants, revealing that the majority (53.8%, $n=14$) function at Level 5, requiring transportation in a wheelchair. This is followed by Level 4 (self-mobility with limitations) at 34.6% ($n = 9$), while the remaining minority consists of Level 2 (walks with limitations) at 7.7% ($n = 2$) and Level 3 (walks using a hand-held mobility device) at 3.8% ($n = 1$).

Table 1. Level of motor disability ($n = 26$).

Level	Frequency	Percent
Level 5 (transported in wheelchair)	14	53.8
Level 4 (self-mobility with limitations)	9	34.6
Level 3 (walks using hand –held mobility device)	1	3.8
Level 2 (walks with limitation)	2	7.7
Total	26	100.0

Figure 1 shows data analysis regarding the clinical classification of the study participants ($n = 26$). It revealed that the quadriplegic type of CP was the most predominant form of CP, observed in 66% ($n = 17$) participants in this study. This was followed by the hemiplegia type, which accounted for 19% ($n = 5$) of the cases. The remaining 15% ($n = 4$) of the sample represented the diplegic type of CP.

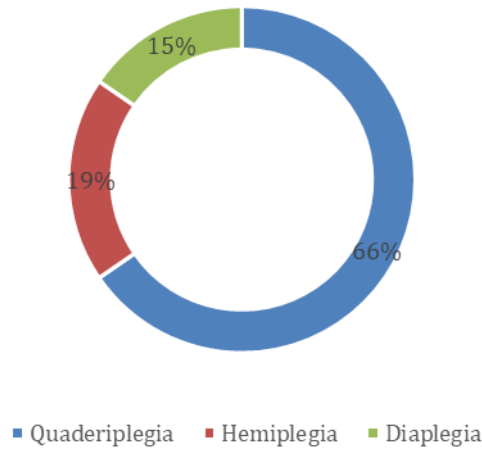


Figure 1. Type of CP (n = 26).

As shown in [Table 2](#), a paired sample t-test (n = 26) analyzing right-side calf spasticity revealed an increase in the mean MAS score from 2.0 pre-treatment to 2.5 post-treatment. The increase in spasticity was not statistically significant (p = 0.916), indicating that the treatment did not produce a measurable reduction in this cohort study.

Table 2. Comparison of the right side pre- and post-treatment for calf spasticity in MAS (n = 26).

Paired Sample T-test					
		Number	MAS		P-value
Variables			Mean	Std. Deviation	
Pair 1	Pre-treatment	26	2.0	1.13	0.916
	Post-treatment	26	2.5	1.74	

Based on the data provided in [Table 3](#), a paired sample t-test was conducted to evaluate the changes in passive ROM for right-side ankle dorsiflexion following treatment. The results show a statistically significant improvement (p = 0.001), with the mean ROM increasing from 12° at pre-treatment to 15° at post-treatment. This indicates that the intervention effectively increased the flexibility and joint mobility of the ankle in the 26 participants studied.

Table 3. Comparison of the right side pre- and post-treatment for ankle dorsiflexion passive ROM (n = 26).

Paired Sample T-test					
		Number	ROM		P-value
Variables			Mean	Std. Deviation	
Pair 1	Pre-treatment	26	12	3.6	0.001
	Post-treatment	26	15	4.8	

As shown in [Table 4](#), a paired sample t-test (n = 26) analyzing left-side calf spasticity revealed an increase in the mean MAS score from 1.9 pre-treatment to 2.3 post-treatment. The increase in spasticity was not statistically significant (p = 0.437), indicating the treatment did not produce a measurable reduction in this cohort study.

Table 4. Comparison of the left side pre- and post-treatment for calf spasticity in MAS (n = 26).

Paired Sample T-test					
;Variables		Number	MAS		P- value
			Mean	Std. Deviation	
Pair 1	Pre-treatment	26	1.9	1.02	0.437
	Post-treatment	26	2.3	1.59	

Based on the data provided in [Table 5](#), a paired sample t-test was conducted to evaluate the changes in passive ROM for left-side ankle dorsiflexion following treatment. The results show a statistically significant improvement ($p = 0.004$), with the mean ROM increasing from 12° at pre-treatment to 15° at post-treatment. This indicates that the intervention effectively increased the flexibility and joint mobility of the ankle in the 26 participants studied.

Table 5. Comparison of the left side pre- and post-treatment for ankle dorsiflexion passive ROM (n = 26).

Paired Sample T-test					
Variables		Number	ROM		P-value
			Mean	Std. Deviation	
Pair 1	Pre-treatment	26	12	3.6	0.004
	Post-treatment	26	15	4.8	

4. Discussion

The study found a significant concentration of motor disability at the more severe end of the functional spectrum, with participants categorized at Levels 4 and 5, which require extensive mobility support or wheelchair transportation. This distribution contrasts with larger population-based registries, where mild impairments at Levels 1 and 2 typically represent the majority, often exceeding 50% of cases ([Hassan Abdelnour et al., 2023](#)). The high prevalence of Level 5 in this cohort is clinically critical, as individuals at this stage exhibit profound limitations in head and trunk control and often rely entirely on caregivers for basic mobility ([Oskoui, Majnemer, et al., 2013](#)). Such severe motor impairment is consistently associated with a significantly higher caregiver burden, increased psychosocial stress, and a greater need for complex assistive technology ([Palisano et al., 2008](#)). Consequently, these results emphasize the urgent need for specialized rehabilitation strategies and robust family support systems tailored to high-dependency populations.

Our findings deviate slightly from large-scale Western registries. For instance, many studies in high-income countries report diplegia or hemiplegia as the most common subtypes, often linked to the increased survival of preterm infants (13). However, the high prevalence of quadriplegia observed in this study aligns more closely with studies conducted in developing regions or specialized tertiary care settings. Research by Oskoui et al. (2013) suggests that in areas where neonatal care may be less accessible or where birth asphyxia remains a primary etiology, the more severe spastic quadriplegic forms of CP tend to be more frequent ([Murugasen, 2022](#)). The dominance of quadriplegia in this study suggests a high burden of disability among the participants. Quadriplegic CP is typically associated with greater Gross Motor Function Classification System (GMFCS) levels, which often require total assistance for mobility. It is also associated with increased comorbidities such as oromotor dysfunction, epilepsy, and cognitive impairment ([Oskoui, Coutinho, et al., 2013](#)). On the other hand, the relatively lower percentage of diplegic cases in our study is a point of interest. In the literature, spastic diplegia is the "hallmark" of prematurity-related brain

injury. The lower frequency here suggests that the underlying etiologies in this specific participant group are more likely related to extensive cortical damage rather than isolated white matter injury (Rosenbaum et al., 2007)

The analysis of calf spasticity in this study demonstrated a numerical increase in mean MAS scores for both the right side and the left side following treatment. However, these changes failed to reach statistical significance. These findings suggest that the intervention did not produce a measurable reduction in muscle tone within this specific group. The lack of significant improvement may be attributed to the inherent limitations of the MAS, which is often criticized for its subjectivity and lack of sensitivity to subtle clinical changes, particularly in the lower extremities (Graham et al., 2016b). Consequently, while the treatment did not demonstrate efficacy in reducing spasticity according to the MAS, future studies should consider incorporating objective measures, such as electromyography or biomechanical analysis, to better capture physiological shifts in muscle resistance (Graham et al., 2016b).

The findings of this study demonstrate that the intervention led to a statistically significant increase in ROM for ankle dorsiflexion bilaterally. The symmetry in improvement across both ankles suggests that the intervention provides a consistent mechanical or neurophysiological effect. The transition to 15° of dorsiflexion moves participants closer to the functional requirements for normal gait, which typically necessitates at least 10° to 15° of flexion during the terminal stance phase. These results align with previous research indicating that targeted manual therapy or stretching interventions can effectively modulate soft tissue extensibility and joint arthrokinematics (Meseguer-Henarejos et al., 2018). Given the significance of these improvements in a cohort of 26 participants, this intervention appears to be a viable strategy for enhancing joint mobility and potentially reducing compensatory movements associated with limited ankle flexibility (Neumann, 2024).

A limitation of this study is the single-group pre–post design, which lacks a counterfactual control group. Consequently, the observed changes cannot be definitively attributed to the intervention, as external factors or natural progression. In addition, while the MAS was chosen for its clinical feasibility, these measurement constraints mean that subtle changes in spasticity may not have been captured, and these specific findings must be interpreted with caution.

5. Conclusion

This exploratory study highlights a high concentration of severe motor disability (GMFCS Levels 4 and 5) and spastic quadriplegia within the cohort, reflecting a significant burden of care and a likely etiology linked to extensive cortical damage. While the intervention did not yield statistically significant reductions in calf spasticity according to MAS scores, a statistically significant increase in bilateral ankle dorsiflexion range of motion was observed. Although these preliminary findings suggest potential for enhancing joint mobility in high-dependency populations, given the exploratory nature of this single-group study and the lack of change in the primary spasticity outcome, these results must be interpreted with caution. Further controlled trials are necessary to rigorously evaluate the efficacy of this strategy.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

References

- Abdelnour, H. M. (2020). Evaluating the feasibility of the expansion of community based rehabilitation into the physiotherapy curriculum in ,ahfad university for women. *University of the Western Cape*. <https://hdl.handle.net/10566/11699>
- Abdullahi, H., Satti, M., Rayis, D. A., Imam, A. M., & Adam, I. (2013). Intra-partum fever and cerebral palsy in Khartoum, Sudan. *BMC Research Notes*, 6(1), 163. <https://doi.org/10.1186/1756-0500-6-163>
- Almaly, A. R., Mashaal, A. H., Hawamdeh, M., Abdelnour, H. M., & Mahmood, S. (2025). *Efficacy of massage and stretching for controlling hamstring muscle spasticity in children with spastic diplegia cerebral palsy: A comparative study*.
- Barnes, M. P., & Johnson, G. R. (Eds). (2008). *Upper Motor Neurone Syndrome and Spasticity: Clinical Management and Neurophysiology* (2nd edn). Cambridge University Press. <https://doi.org/10.1017/CBO9780511544866>
- Graham, H. K., Rosenbaum, P., Paneth, N., Dan, B., Lin, J.-P., Damiano, D. L., Becher, J. G., Gaebler-Spira, D., Colver, A., Reddihough, D. S., Crompton, K. E., & Lieber, R. L. (2016a). Erratum: Cerebral palsy. *Nature Reviews Disease Primers*, 2(1), 16005. <https://doi.org/10.1038/nrdp.2016.5>
- Graham, H. K., Rosenbaum, P., Paneth, N., Dan, B., Lin, J.-P., Damiano, D. L., Becher, J. G., Gaebler-Spira, D., Colver, A., Reddihough, D. S., Crompton, K. E., & Lieber, R. L. (2016b). Erratum: Cerebral palsy. *Nature Reviews Disease Primers*, 2(1), 16005. <https://doi.org/10.1038/nrdp.2016.5>
- Hassan Abdelnour, Abuelgasim Mohamed, Duaa El Tigani, & Balsam Abdallha. (2023). Physical Disabilities and Rehabilitation Services in Khartoum State, Sudan. *The International Journal of Humanities & Social Studies*. <https://doi.org/10.24940/theijhss/2023/v11/i9/HS2309-016>
- Horsch, A., Klotz, M. C. M., Platzer, H., Seide, S., Zeaiter, N., & Ghandour, M. (2021). Is the Prevalence of Equinus Foot in Cerebral Palsy Overestimated? Results from a Meta-Analysis of 4814 Feet. *Journal of Clinical Medicine*, 10(18), 4128. <https://doi.org/10.3390/jcm10184128>
- Kruse, A., Habersack, A., Jaspers, R. T., Schrapf, N., Weide, G., Svehlik, M., & Tilp, M. (2022). Acute Effects of Static and Proprioceptive Neuromuscular Facilitation Stretching of the Plantar Flexors on Ankle Range of Motion and Muscle-Tendon Behavior in Children with Spastic Cerebral Palsy—A Randomized Clinical Trial. *International Journal of Environmental Research and Public Health*, 19(18), 11599. <https://doi.org/10.3390/ijerph191811599>
- Meseguer-Henarejos, A.-B., Sánchez-Meca, J., López-Pina, J.-A., & Carles-Hernández, R. (2018). Inter- and intra-rater reliability of the Modified Ashworth Scale: A systematic review and meta-analysis. *European Journal of Physical and Rehabilitation Medicine*, 54(4). <https://doi.org/10.23736/S1973-9087.17.04796-7>
- Murugasen, S. (2022). *A systematic review of cerebral palsy in African paediatric populations* [Stellenbosch : Stellenbosch University]. <http://hdl.handle.net/10019.1/126157>
- National Institute of Neurological Disorders and Stroke. (2023). <https://www.ninds.nih.gov/>
- Neumann, D. A. (2024). *Neumann's Kinesiology of the Musculoskeletal System-E-Book: Neumann's Kinesiology of the Musculoskeletal System-E-Book*. Elsevier Health Sciences.
- Oskoui, M., Coutinho, F., Dykeman, J., Jetté, N., & Pringsheim, T. (2013). An update on the prevalence of cerebral palsy: A systematic review and meta-analysis. *Developmental Medicine & Child Neurology*, 55(6), 509–519. <https://doi.org/10.1111/dmcn.12080>
- Oskoui, M., Majnemer, A., Dagenais, L., & Shevell, M. I. (2013). The Relationship Between Gross Motor Function and Manual Ability in Cerebral Palsy. *Journal of Child Neurology*, 28(12), 1646–1652. <https://doi.org/10.1177/0883073812463608>
- Palisano, R. J., Rosenbaum, P., Bartlett, D., & Livingston, M. H. (2008). Content validity of the expanded and revised Gross Motor Function Classification System. *Developmental Medicine & Child Neurology*, 50(10), 744–750. <https://doi.org/10.1111/j.1469-8749.2008.03089.x>

- Rosenbaum, P., Paneth, N., Leviton, A., Goldstein, M., Bax, M., Damiano, D., Dan, B., & Jacobsson, B. (2007). A report: The definition and classification of cerebral palsy April 2006. *Dev Med Child Neurol Suppl*, 109(suppl 109), 8–14.
- Satti, L., Abdelnour, H., Alawad, S., Gabrallah, M., & Alshiekh, R. (2026). Prevalence of Stress and Neck Pain Among Accountants: A Cross-Sectional Study. *European Journal of Medical and Health Research*, 4(1), 156–161. [https://doi.org/10.59324/ejmhr.2026.4\(1\).22](https://doi.org/10.59324/ejmhr.2026.4(1).22)



Barriers to Injury Prevention in University-Level Contact Sports

Haithem Nasr^{1,2} * , Meftah Madi³, Mastoura Aksh⁴, and Hassan Abdelnour^{5,6} 



¹ Department of Physiotherapy, Faculty of Community and Health Sciences, University of the Western Cape, Cape Town, South Africa.

² Department of Physiotherapy, Tawargha General Hospital, Tawargha, Libya.

³ Department of Biomedical Sciences, Cape Peninsula University of Technology, Cape Town, South Africa.

⁴ Department of Family and Community Medicine, University of Benghazi, Benghazi, Libya.

⁵ Department of Rehabilitation Sciences, College of Medical Sciences, Northern Border University, Arar, KSA.

⁶ Department of Physiotherapy, School of Health Sciences, Ahfad University for Women, Omdurman, Sudan.

ARTICLE INFO

Received: 05th March 2026

Received in revised form: 01st June 2026

Accepted: 04th June 2026

Published: 15th September 2026

*Corresponding Author:

Haithem Nasr

E-mail :

hmarshy1789@gmail.com

Citation : Nasr, H, Madi, M, Aksh, M, & Abdelnour, H. (2026) Barriers to Injury Prevention in University-Level Contact Sports. *Modern Journal of Health and Applied Sciences*, 3(2), 09-16.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Injuries affecting the musculoskeletal system impact over 40% of active individuals annually. In university settings, athletes face high risks during matches especially in contact sports like football where injury rates can reach 36 per 1,000 hours. While evidence-based strategies like neuromuscular training and proper warm-up/cool-down routines can reduce injury risk by 50%, implementation remains inconsistent.

This study aimed to explore the knowledge and adoption of injury prevention strategies among student-athletes and to identify barriers to their implementation. A descriptive cross-sectional study design was conducted between September 2016 and September 2017 with 101 student-athletes at UWC using purposive and convenience sampling. Data were collected via validated questionnaires focusing on sociodemographic and preventive practices. Analysis was performed using SPSS version 24.

The sample was predominantly aged 20 – 24 (58.4%), with 1–2 years of experience (65.6%). Football was the most common sport (49.5%). While players recognised doctors/physiotherapists (72.4%) and coaches (50%) as vital knowledge sources, practical application was hindered by structural barriers. Specifically, 59% of athletes skipped warm-ups due to lack of time, and 31% neglected cool-downs due to exhaustion.

Despite trusting medical advice, university athletes skip injury prevention due to time constraints and exhaustion.

Keywords: Injuries, Contact sports, Prevention, Warm-up, Cool-down, University of the Western Cape (UWC).

1. Introduction

Injuries involve harm to the body, predominantly affecting the musculoskeletal system, and typically occur during sports, athletic endeavors, or exercise. They can vary in severity, spanning from sudden injuries to long-term conditions stemming from overuse. Such injuries often lead to pain, inflammation, or a temporary disruption of normal function. Sports injuries are quite common, with research showing that over 40% of active individuals sustain an injury each year, varying by sport and activity level (Chowdhery et al., 2025). These injuries often involve the knee, ankle, or shoulder and are typically the result of overuse or acute trauma. The incidence is notably higher in contact sports such as football. Football players experience an average injury rate of 8.1 injuries per 1,000 hours of play, which rises significantly to 36 injuries per 1,000 hours during matches (López-Valenciano et al., 2020). The most frequent types of injuries involve muscles and tendons, accounting for 39.78% to 44.56% of all injuries, with the majority occurring in the lower extremities. The likelihood of injury during matches is almost ten times greater than during training sessions (López-Valenciano et al., 2020). Prevention strategies, such as neuromuscular training, have been shown to reduce sports injuries by an estimated 50% (*STOP Is a Valuable Tool for Local Community and Coaches* | AOSSM, 2023). Preventing sports injuries involves a balanced approach that combines thorough preparation, correct techniques, and adherence to safety protocols. Important practices include starting with dynamic stretching to warm up, strengthening core muscles for better stability, wearing suitable protective gear, incorporating adequate rest and recovery into your routine, and progressively building up the intensity of your workouts.

Various strategies are implemented in sports to reduce the prevalence of injuries. Warm-up strategies in sports play a vital role in preventing injuries, reducing risks through promoting better blood circulation, enhancing tissue flexibility, and improving joint lubrication (*Warm-Up Exercises for Injury Prevention* | Precision Performance, 2025). Raising muscle temperature and boosting mental focus through proper warm-up routines, particularly dynamic stretching, help protect against strains, sprains, and ligament damage. Furthermore, cooling down after physical activity can significantly lower the risk of injuries. It achieves this by gradually bringing down the heart rate, preventing blood from pooling in the extremities, and easing muscle stiffness. Additionally, it helps flush out lactic acid, reduce the chances of dizziness post-exercise, and support improved flexibility (Van Hooren & Peake, 2018).

The University of the Western Cape (UWC), situated in the Bellville suburb of Cape Town, South Africa (Abdelnour & Twetwa, 2008), manages a comprehensive sports infrastructure that supports different sporting codes, ranging from recreational to high-performance levels. It offers a comprehensive system for overseeing sports clubs and addressing sports injuries, supported by its specialized sports department and academic research teams. Finally, the study investigates the levels of knowledge and adoption of injury prevention strategies among student-athletes, as well as the barriers to their implementation.

2. Methodology

A descriptive cross-sectional study design was used. The study was carried out at the UWC in Cape Town, South Africa. The study population consisted of athletes who were registered with sports clubs at UWC. Both purposive and convenience sampling methods were utilized. The study included a total of 101 students.

To achieve the aim of the study, questionnaires developed by Nuhu, A. (Nuhu, 2008) for a similar study in Rwanda, titled “Factors Influencing Implementation of Soccer Injury Prevention Strategies in Rwanda,” were used for data collection. The questionnaires included questions designed to evaluate sociodemographic characteristics and the adoption of preventive strategies. In addition, they included questions regarding knowledge, attitude, and practices related to injury prevention. A pilot study was conducted with 15 players from various sports clubs at UWC, who were not part of the main study. Each participant took 15 minutes to complete the questionnaires, and no issues were reported.

The study duration was between September 2016 and September 2017. During the data collection period, questionnaires were distributed before the start of each training session at the UWC sport department. The Statistical Package for the Social Sciences (SPSS), version 24, was used for descriptive data analysis. Approval to conduct the study was obtained from the University of the Western Cape Humanities and Social Sciences Research Ethics Committee (Ethics Reference Number: HS 16/5/44). The Manager of Student Administration at UWC approved the inclusion of students in the study. The researcher distributed consent letters to the participants, asking them to voluntarily take part in the study.

3. Results

The study population consisted of 101 undergraduate participants. As shown in [Table 1](#), the majority of respondents fell within the 20-24 age group (58.4%), followed by those aged 14-19 (29.7%).

Table 1. Age.

Age	N	%
14-19	30	29.70
20-24	59	58.40
25-29	12	11.90
Total	101	100

In [Table 2](#), a significant portion of the sample is relatively new to their sport, with 65.6% having 1-2 years of experience, while only one participant reported 7 or more years in the field.

Table 2. Years of experience

Years	Frequency	%
1-2	66	65.6
3-4	28	27.7
5-6	6	5.9
≥ 7	1	1
Total	101	100

Of the types of sport, football was identified as the most prevalent sport among participants, accounting for 53.4% of the total, followed by Basketball (18.8%). Cricket and Rugby represented 13.8% of the sample ([Table 3](#)).

Table 3. Types of sports

Sport	N	%
Rugby	14	13.8
Football	54	53.4
Basketball	19	18.8
Cricket	14	13.8
Total	101	100

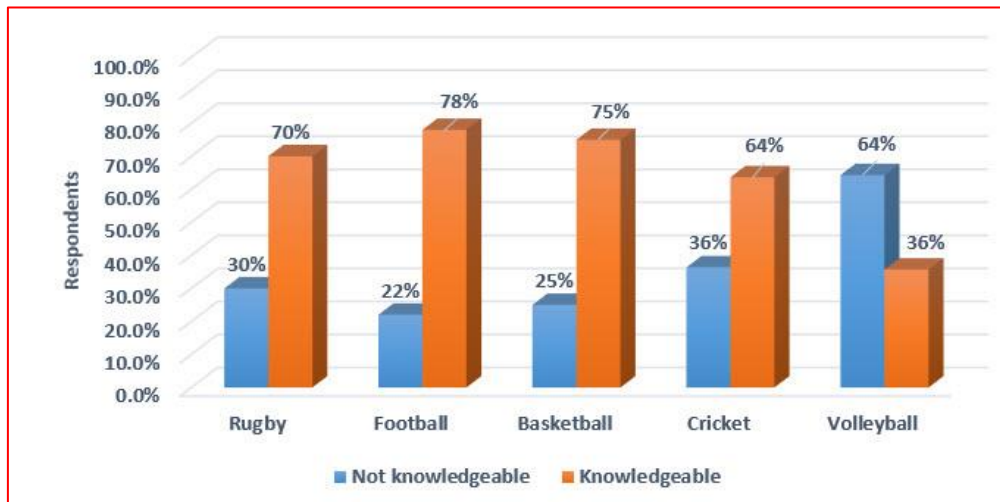


Figure 1. Players' Knowledgeability of Injury Prevention Within the Type of Sports.

Figure 1, illustrates the levels of knowledgeability regarding injury prevention across the different sporting disciplines. To acquire this knowledge, players rely on various figures.

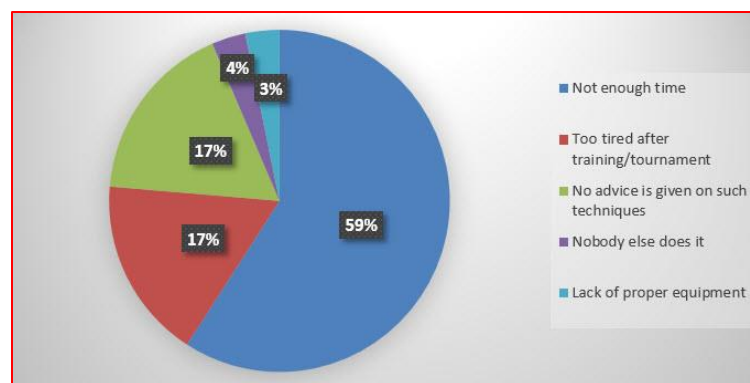


Figure 2. Players' Reasons for Not Doing Warm-up Before Training as an Injury Prevention Strategy.

As shown in **Figure 2**, 60% of the players do not have enough time to do a warm-up before the training, while 17% did not get any advice to do so. Only 3% lacked proper equipment, and 4% did not do a warm-up before training because nobody else was doing it.

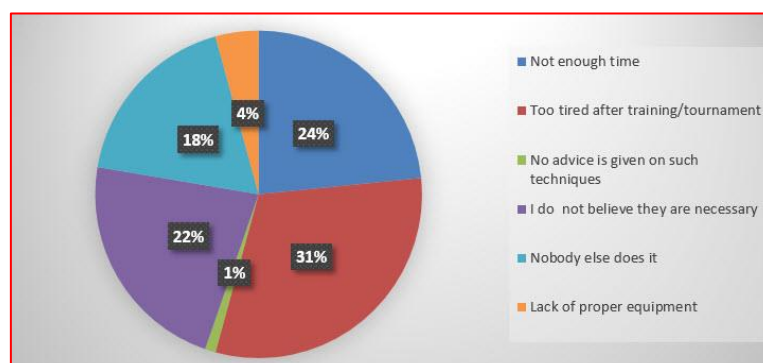


Figure 3. Players' Reasons for Not Doing Cool-down After Training as an Injury Prevention Strategy.

As shown in [Figure 3](#), during training, 32% of the players did not do a cool-down because they were tired after training. Some players indicated lack of enough time (24%), do not believe it is necessary (22%), nobody else does it (18%), lack of proper equipment (4%) and no advice was given as reasons why they did not cool down after training.

In [Table 4](#), doctors and physiotherapists are viewed as the most vital source, with 72.4% of players ranking them as "most important" Source. Coaches also play a significant role, rated as "most important" by 50% of the players. Conversely, media was largely regarded as the "least important" source (65.3%).

Table 4. Sources of Players' Knowledge of Injury Prevention.

Learned about injury prevention		Frequency	Percentage
Doctor / Physiotherapist	Most important	71	72.4
	Important	24	24.5
	Least important	3	3.1
Media	Most important	8	8.2
	Important	26	26.5
	Least important	64	65.3
Coaches	Most important	49	50.0
	Important	44	44.9
	Least important	5	5.1

4. Discussion

The participant profile predominantly comprised individuals aged between 14 and 24 years, with a significant concentration in the 20–24-year age group. This distribution aligns with the traditional university-aged population in South Africa.

The sample indicates a concentration of athletes in the nascent stages of their competitive careers, with most participants reporting only one to two years of experience. This suggests that university sports clubs function primarily as foundational environments for athletic initiation rather than as hubs for long-term professional specialisation. These findings are consistent with the "initiation" and "development" phases of the holistic athletic career model, wherein student-athletes explore diverse sporting disciplines during their transition into higher education ([Wylleman, 2019](#)). The high prevalence of football among participants underscores its status as a cornerstone of university sporting culture. This dominance mirrors global trends, in which football is recognised as a "universal sport" characterised by low economic barriers and minimal equipment requirements, enhancing accessibility for diverse student cohorts ([Giulianotti, 2015](#)). Within the university ecosystem, football serves as a critical mechanism for social cohesion, fostering institutional pride and community engagement across academic and cultural divides. The sport's adaptability—spanning informal recreational play to structured competitive leagues further explains its disproportionate representation relative to more specialised athletic disciplines.

The data demonstrate a definitive hierarchy regarding the perceived credibility of information sources for injury prevention. The overwhelming reliance on physicians and physiotherapists as the most critical sources aligns with evidence suggesting that student-athletes prioritise clinical expertise to safeguard their "dual-career" longevity ([Bolling et al., 2020](#)). The professionalisation of university support staff has cultivated a culture in which athletes seek medical validation to manage the physiological demands of competition. While coaches are viewed as a "first line of defence," their secondary ranking behind medical professionals suggests that athletes distinguish between performance-based instruction and clinical safety protocols.

Coaches remain pivotal in the knowledge translation process; although they may lack clinical credentials, their role in implementing preventive measures during daily training is essential to adherence (Geertsema et al., 2021). Conversely, the dismissal of media as a credible source reflects a critical scepticism toward unverified sports health trends and the "infodemic" of digital misinformation (Wang et al., 2026).

This preference for face-to-face institutional expertise suggests that centralised, expert-led prevention programmes are likely to achieve higher compliance than decentralised digital campaigns (Whalan et al., 2020). The data identify "time pathology" as the primary systemic barrier to injury prevention, with participants frequently citing insufficient time for warm-up protocols. This is a critical concern, as truncated or omitted warm-ups significantly escalate the risk of acute soft-tissue injuries (Ding et al., 2022). In the university context, the pressure to balance rigorous academic schedules with limited facility availability often results in "temporal crowding," where tactical training is prioritised over physiological (Whalan et al., 2020). Furthermore, reports of physical exhaustion and a lack of professional guidance indicate deficiencies in technical supervision and workload management. The absence of coach-led warm-up directives reflects a deficit in the organisational "safety culture" (Read et al., 2018). When athletes are too fatigued to engage in preparatory exercises, this suggests a failure to monitor internal training loads, which increases vulnerability to overtraining and injury (Soligard et al., 2016). Because these barriers appear to be structural and educational rather than material, university programmes must transition toward mandatory Integrated Neuromuscular Training (INT) protocols that are strictly embedded within the allocated training duration (Bolling et al., 2018).

5. Limitation

The study's small sample from a single institution limits generalisability, while its exclusive reliance on descriptive statistics prevents hypothesis testing. Adding inferential analysis and a broader cohort would strengthen the paper.

6. Conclusion

This study shows that university athletes are mostly young beginners, with football dominating the sports culture. While players highly trust doctors and physiotherapists for injury-related advice, a major gap exists between their knowledge and actual practices. Severe time constraints and physical exhaustion act as the primary structural barriers, forcing athletes to skip critical warm-up and cool-down protocols. Since these issues stem from poor scheduling and workload management rather than a lack of equipment, university clubs must transform their safety culture. Moving forward, sports administrations must integrate mandatory, expert-led neuromuscular training directly into standard practice times. Finally, coaches must receive better training to monitor player fatigue and enforce safety compliance in coordination with medical staff.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

Acknowledgments

The author thanks the UWC Sports Department for their support and permission to conduct this study. Sincere gratitude is also extended to the student athletes for their essential participation and honesty.

References

- Abdelnour, H., & Twetwa, A. (2008). Prevalence and pattern of injuries among players at the university of the Western Cape volleyball club. <https://uwcscholar.uwc.ac.za/items/9607ce1d-6e2f-4255-8701-62bf384993af>
- Bolling, C., Delfino Barboza, S., van Mechelen, W., & Pasman, H. R. (2020). Letting the cat out of the bag: Athletes, coaches and physiotherapists share their perspectives on injury prevention in elite sports. *British Journal of Sports Medicine*, 54(14), 871–877. <https://doi.org/10.1136/bjsports-2019-100773>
- Bolling, C., Van Mechelen, W., Pasman, H. R., & Verhagen, E. (2018). Context Matters: Revisiting the First Step of the ‘Sequence of Prevention’ of Sports Injuries. *Sports Medicine*, 48(10), 2227–2234. <https://doi.org/10.1007/s40279-018-0953-x>
- Chowdhery, A., Agarwal, A., Saini, A., & CHOWDHERY, A. (2025). Prevalence of sports injuries among athletes in the Lucknow region: A cross-sectional study. *Cureus*, 17(8). <https://www.cureus.com/articles/400208-prevalence-of-sports-injuries-among-athletes-in-the-lucknow-region-a-cross-sectional-study.pdf>
- Ding, L., Luo, J., Smith, D. M., Mackey, M., Fu, H., Davis, M., & Hu, Y. (2022). Effectiveness of Warm-Up Intervention Programs to Prevent Sports Injuries among Children and Adolescents: A Systematic Review and Meta-Analysis. *International Journal of Environmental Research and Public Health*, 19(10), 6336. <https://doi.org/10.3390/ijerph19106336>
- Geertsema, C., Geertsema, L., Farooq, A., Harøy, J., Oester, C., Weber, A., & Bahr, R. (2021). Injury prevention knowledge, beliefs and strategies in elite female footballers at the FIFA Women’s World Cup France 2019. *British Journal of Sports Medicine*, 55(14), 801–806.
- Giulianotti, R. (2015). *Sport: A critical sociology*. John Wiley & Sons. [https://books.google.com/books?hl=fr&lr=&id=7DpcCwAAQBAJ&oi=fnd&pg=PT5&dq=Giulianotti,+R.+\(2023\).+Sport:+A+critical+sociology+\(2nd+ed.\).+Polity+Press.+www.politybooks.com+&ots=cfPbTCOVcp&sig=Kkb0OWR R8bL7-P6nfifbl8tpwnE](https://books.google.com/books?hl=fr&lr=&id=7DpcCwAAQBAJ&oi=fnd&pg=PT5&dq=Giulianotti,+R.+(2023).+Sport:+A+critical+sociology+(2nd+ed.).+Polity+Press.+www.politybooks.com+&ots=cfPbTCOVcp&sig=Kkb0OWR R8bL7-P6nfifbl8tpwnE)
- López-Valenciano, A., Ruiz-Pérez, I., Garcia-Gómez, A., Vera-Garcia, F. J., De Ste Croix, M., Myer, G. D., & Ayala, F. (2020). Epidemiology of injuries in professional football: A systematic review and meta-analysis. *British Journal of Sports Medicine*, 54(12), 711–718.
- Nuhu, A. (2008). Factors influencing implementation of soccer injury prevention strategies in Rwanda [PhD Thesis, University of the Western Cape]. <https://uwcscholar.uwc.ac.za/items/814e4d1a-eda1-4405-bd79-d9de7650df28>
- Read, P. J., Jimenez, P., Oliver, J. L., & Lloyd, R. S. (2018). Injury prevention in male youth soccer: Current practices and perceptions of practitioners working at elite English academies. *Journal of Sports Sciences*, 36(12), 1423–1431.
- Soligard, T., Schweltnus, M., Alonso, J.-M., Bahr, R., Clarsen, B., Dijkstra, H. P., Gabbett, T., Gleeson, M., Häggglund, M., & Hutchinson, M. R. (2016). How much is too much?(Part 1) International Olympic Committee consensus statement on load in sport and risk of injury. *British Journal of Sports Medicine*, 50(17), 1030–1041.
- STOP is a Valuable Tool for Local Community and Coaches | AOSSM. (2023). <https://www.sportsmed.org/membership/sports-medicine-update/summer-2023/stop-is-a-valuable-tool-for-local-community-and-coaches>
- Van Hooren, B., & Peake, J. M. (2018). Do We Need a Cool-Down After Exercise? A Narrative Review of the Psychophysiological Effects and the Effects on Performance, Injuries and the Long-Term Adaptive Response. *Sports Medicine*, 48(7), 1575–1595. <https://doi.org/10.1007/s40279-018-0916-2>
- Wang, H., Guo, G., Yang, M., Jiang, S., Yang, W., & Zhao, Y. (2026). Social Media as a Double-Edged Sword in Sports Nutrition Dissemination: Opportunities, Challenges, and Pathways Forward. *Current Nutrition Reports*, 15(1), 14. <https://doi.org/10.1007/s13668-026-00735-7>

- Warm-Up Exercises for Injury Prevention | Precision Performance. (2025, August 20). <https://precisionperformancept.com/the-importance-of-warm-up-key-to-injury-prevention/>
- Whalan, M., Lovell, R., & Sampson, J. A. (2020). Do Niggles Matter? - Increased injury risk following physical complaints in football (soccer). *Science and Medicine in Football*, 4(3), 216–224. <https://doi.org/10.1080/24733938.2019.1705996>
- Wylleman, P. (2019). A developmental and holistic perspective on transiting out of elite sport. <https://psycnet.apa.org/record/2019-05400-011>



Harnessing Bioinformatics to Decode Drug Resistance and Guide Vaccine Design in *Mycobacterium tuberculosis*

Ahmed Mohammed Hayawi ^{1*} 



¹ Department of Microbiology, College of Medicine, University of Mosul, Mosul.

ARTICLE INFO

Received: 29th April 2026
Received in revised form: 13th June 2026
Accepted: 16th June 2026
Published: 15th September 2026

*Corresponding Author:

Ahmed Mohammed Hayawi
E-mail :
ahmadhay@uomosul.edu.iq

Citation: Hayawi. A. M (2026). Harnessing Bioinformatics to Decode Drug Resistance and Guide Vaccine Design in *Mycobacterium tuberculosis*. *Modern Journal of Health and Applied Sciences*, 3(2), 17-30.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Mycobacterium tuberculosis presents a major challenge to global health due to tuberculosis (TB), which is worsened by the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains. This study provides a thorough examination of bioinformatics research, highlighting how the integration of genomic, proteomic and metabolomic data into TB research has revolutionised the way TB is studied, particularly in understanding resistance mechanisms, identifying new therapeutic targets, and expediting the development of diagnostic tools and vaccines. Whole-genome sequencing (WGS) and newer technologies like Oxford Nanopore Technologies (ONT) have since paved the way to the rapid identification of strain-specific mutations, with novel bioinformatics tools like PhyResSE, Mykrobe and GBOOST offering a powerful platform in mutation profiling, resistance prediction and strain classification. Furthermore, resistance prediction is also being transformed by machine learning and AI-based models, which improve the accuracy of clinical choices and monitoring options. Epitope prediction, molecular docking, and analysis of population coverage to inform the selection of promising immunogenic candidates are also proposed in the study as an in silico pipeline of rational vaccine design. Although continued efforts at infrastructure, standardisation, and workforce training are always ongoing in many settings where this is still seen as a major issue, especially in high-burden settings, this paradigm shift in an interdisciplinary approach is a clear indication of progress in the fight against one of the most prevalent and persistent infectious diseases in the world.

Keywords: Bioinformatics, Drug resistance, Epitope-based vaccine design, Tuberculosis genomics.

1. Introduction

1. 1. *Mycobacterium tuberculosis*'s Global Health Impact

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* and is one of the most dangerous infectious diseases in the world. TB is one of the key health challenges that are faced globally, mainly because of the high mortality rates in developing countries. The infection spreads through respiratory droplets and is difficult to contain, especially with the emergence of drug-resistant strains. Recent developments include the emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains, which are difficult to treat, in addition to acting as a fast spreader of the epidemic. Studies have shown that one of the main factors behind further infection and mortality rates is antibiotic resistance, leading to the failure of conventional interventions and increased spread of the populations (Couvin et al., 2025). This issue poses a global health challenge and requires innovative interventions that integrate biology and computational sciences to improve treatment and diagnostics. Also, TB has a significant health system burden and is a major drain on national economies, particularly where healthcare infrastructure is weak (Acharya et al., 2020). The World Health Organization (WHO) Global Tuberculosis Report 2023 states that in 2022, TB claimed 1.3 million lives and infected 7.5 million people worldwide, ranking it as the second deadliest infectious disease after COVID-19.

1. 2. Bioinformatics Contribution in the Study of *M. tuberculosis*

Bioinformatics is the use of information technology, especially computational methods, in the collection, analysis and interpretation of biological information, particularly genomic and proteomic sequences. Innovations in genomics and biological databases have bridged knowledge gaps in *M. tuberculosis* biology and genetic differences. Whole-genome sequencing (WGS) of *M. tuberculosis* has opened new opportunities in the study of the genetic makeup of various forms, which has led to a better understanding of biology as well as resistance to drugs. Bioinformatics tools play an important part in the identification of the mutations that are associated with drug resistance and in the identification of the potential drug targets, including proteins involved in resistance or virulence (Sola et al., 2001). Additionally, the combination of genomic data with proteomics has greatly benefited research on such complex microorganisms as *M. tuberculosis*, providing both pangenomic and proteomic global perceptions to design strategies focused on diagnosis, therapy, and vaccine development (Alaridah et al., 2019).

1. 3. Objectives and Scientific Significance of the Research

This paper addresses research investigating the potential of bioinformatics technologies in fighting TB, especially with the increasing cases of drug-resistant strains. The main aim is to attain a combined perspective of the genome and proteome of *M. tuberculosis* in order to discover new resistance mechanisms and identify effective drug targets which will be harder to resist in future. Genomic and proteomic platforms can be used together to develop a superior platform for developing specific solutions, such as diagnostics that are faster and the development of new vaccines that offer better preventive measures (Bakuła, Dziurzyński, et al., 2023). Moreover, the study also points out discoveries in the field of diagnosis, treatment, and vaccine development using modern computational analysis, claiming that it may be an effective tool for overcoming the current difficulties that TB and its resistance cause (Rosenthal et al., 2017). This article is a narrative review which summarizes literature from PubMed, Scopus, and Web of Science with the following keywords: bioinformatics, tuberculosis, drug resistance, genomics, vaccine design, and machine learning.

2. Methods of Genome Sequencing and *M. tuberculosis* Data

2. 1. Technologies of Genetic Sequencing Utilised

Genome sequencing has made exponential strides in recent years, resulting in the fast and accurate retrieval of complete genomes of *M. tuberculosis* strains. Some of the revolutionary methods include technologies like WGS and ONT. ONT offers long-read sequencing, which simplifies the identification of tricky genetic mutations, especially in repetitive genomic regions that were previously inaccessible to short-read sequencing technologies. Moreover, ONT minimizes the time of analysis and diagnosis of drug resistance and response to treatment, which enables the process to take hours instead of weeks (Bellad et al., 2022). Contemporary sequencing matches volume with quality, as data quality evaluations aim to obtain definitive genetic data. These developments allow for the rapid and accurate identification of drug-resistant strains through identification of resistance-linked mutations (Bogaerts et al., 2021).

2. 2. Bioinformatics Software and Tools for Genomic Data Analysis

Bioinformatics tools are important and are used in interpreting sequencing data of *M. tuberculosis*. They are used for mutation detection, strain classification and epidemiological studies. Among the well-known tools are PhyResSE, which has an intuitive interface, allows the use of high-grade sequence data, and maintains offline functionality; and Mykrobe, which also has the possibility of offline usage and an intuitive interface to distinguish bacterial species and drug resistance (Cancino-Muñoz et al., 2022). SpolPred software can predict strain types directly in raw sequence reads with higher speed and accuracy than assembly-based strategies, allowing both phylogenetic and epidemiological classification (Chen et al., 2022). Targeted databases such as MUBII-TB-DB contain resistance-related mutations and their biological explanations and help in effective targeting and surveillance of resistance that is evolving (Bakuła, Marczak, et al., 2023). **Table 1** presents bioinformatics tools used in genomic analysis.

Table 1. Major Bioinformatics Tools Used for Genomic Analysis of *M. tuberculosis*.

Tool	Main Function	Algorithm/Method	Input Data Type	Output	Key Features
PhyResSE	Prediction of drug resistance and lineage identification	Reference-based mutation analysis	Raw reads or assembled genomes	Resistance profile and lineage classification	Web-based interface and curated mutation database
Mykrobe	Species identification and drug resistance prediction	De Bruijn graph approach	Raw reads	Drug susceptibility prediction	Rapid analysis and offline capability
SpolPred	In silico spoligotype determination	Spoligotyping algorithm	Raw reads	Strain classification	Fast and highly accurate strain typing

TB-Profiler	Detection of resistance mutations and lineage assignment	Mapping-based variant calling	Raw reads	Drug resistance report and lineage information	Supports MDR/XDR prediction and comprehensive mutation database
MTBseq	Whole genome sequence analysis	Reference-guided SNP analysis pipeline	Raw reads	SNP identification, phylogeny, and resistance profile	High-resolution genomic analysis
FastQC	Sequence quality assessment	Statistical quality control analysis	Raw reads	Sequence quality reports and metrics	Detection of low-quality reads and GC bias
BaseClear pipeline	Genome assembly and variant analysis	Hybrid assembly and mapping approaches	Raw reads	Genome assembly and SNP detection	Suitable for large-scale sequencing projects
MUBII-TB-DB	Annotation of resistance-associated mutations	Database-driven mutation annotation	Assembled genomes and mutation lists	Functional interpretation of mutations	Curated resistance mutation database

A three-dimensional visualisation of the workflow of the pipeline needed in tuberculosis research, which involves all parts of clinical sampling, genome sequencing, bioinformatics systems, and AI-powered prognoses to assist in the diagnosis, therapeutics of the disease or its cure, and disease surveillance, is illustrated in **Figure 1**.

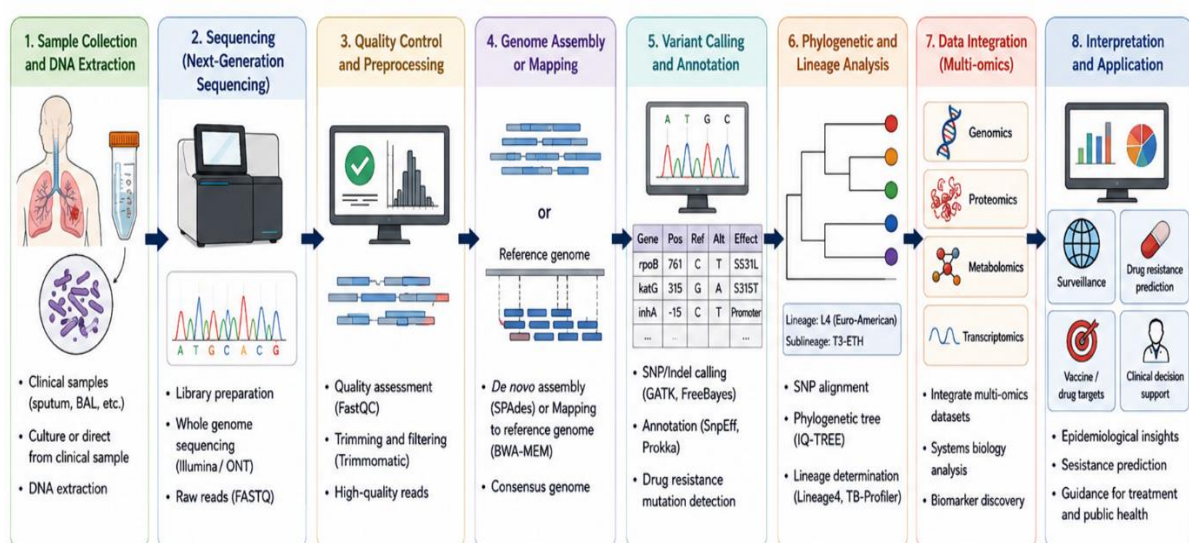


Figure 1. Integrated Bioinformatics Pipeline for *M. tuberculosis* Analysis.

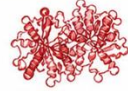
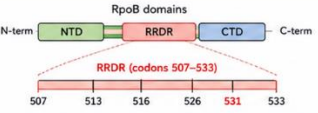
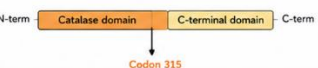
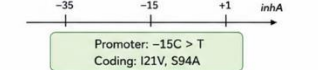
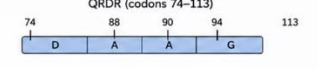
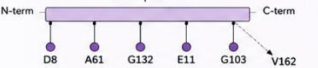
2. 3. Challenges and Opportunities in Clinical and Epidemiological Application of Genome Sequencing

Genome sequencing has witnessed great progress, but its application in clinical and epidemiological practice still has difficulties. These weaknesses are related to the trouble detecting resistance caused by mutations that are not known or by an emergence of new mechanisms. It is also equally inaccurate to distinguish between true resistance-associated mutations and neutral variants. However, there are prospects for expanding early detection through a combination of sequencing data with proteomics and multi-dimensional data, such as gene and protein expression, as well as increasing the detection capacity of resistant cases and monitoring disease outbreaks (Puustinen et al., 2003). The scope of multilayered data analysis creates new horizons in the knowledge of the process of adapting bacteria to antibiotics and creating prescribed treatments (Couvin et al., 2020).

3. Drug Resistance-Associated Mutation Analysis of *M. tuberculosis*

3. 1. Major Genes that Participate in Antibiotic Resistance

The major relationship between drug resistance in *M. tuberculosis* and mutations in certain genes lies in their role as important targets of antibiotics. The key among them are *rpoB* (rifampicin resistance), *katG* and *inhA* (isoniazid resistance), *gyrA* (fluoroquinolones resistance), and *pncA* (pyrazinamide resistance) genes. These genes influence bacterial survival by changing the structure of drug targets, thereby making antibiotics less effective. It is also important to note that such mutations as the one at codon 531 in *rpoB* are powerful indicators of rifampicin resistance and these are among the most frequently reported mutations associated with treatment failure. The importance of comprehending interactions between several mutations lies in the fact that synergetic effects may establish a complex pattern of resistance that restricts therapeutic options (Gautam et al., 2018). A 3D image of the major areas of resistance genes (*rpoB*, *katG*, *inhA*, *gyrA*, *pncA*), the most frequent associated mutations causing drug resistance in tuberculosis, and also their connection to corresponding antibiotics is shown in Figure 2.

Gene	Protein / Target	Location of Major Mutation	Common Mutations	Antibiotic Affected (Mechanism)
<i>rpoB</i> (RNA polymerase β subunit)	 RNA polymerase β subunit		• S531L • H526Y/D • D516V • Q513H • L511P	Rifamycins (Rifampicin)  Inhibits RNA polymerase
<i>katG</i> (Catalase-peroxidase)	 Catalase-peroxidase		• S315T • S315N • S315I	Isoniazid (INH)  Prodrug activated by KatG
<i>inhA</i> (Enoyl-ACP reductase)	 Enoyl-ACP reductase		• -15C > T (promoter) • I21V • S94A	Isoniazid (INH) & Ethionamide (ETH)  Inhibits mycolic acid synthesis
<i>gyrA</i> (DNA gyrase A subunit)	 DNA gyrase A		• D94G • D94A • A90V • G88C/D	Fluoroquinolones (Levofloxacin, Moxifloxacin)  Inhibits DNA gyrase
<i>pncA</i> (Pyrazinamidase)	 Pyrazinamidase		• D8A/G • C9R • E11Q • G132D • V162M	Pyrazinamide (PZA)  Prodrug requiring activation by PncA

Abbreviations: INH: Isoniazid PZA: Pyrazinamide ETH: Ethionamide RRDR: Rifampicin Resistance-Determining Region QRDR: Quinolone Resistance-Determining Region

Figure 2. Major Genes Associated with Antibiotic Resistance in *M. tuberculosis*.

3. 2. Bioinformatics Methods of Detecting and Analyzing Simultaneous Mutations

Single mutations do not usually cause resistance but interactions between multiple mutations of the genes or SNP pairs. More advanced bioinformatics methods, such as GBOOST, can examine such confounding interactions to identify gene-gene combinations that lead to resistance patterns that cannot be explained by single mutations. These studies have also highlighted

the importance of gene families such as PPE proteins, which could have a remote effect on resistance and bacterial adaptation to drug pressure, thereby aiding in the improvement of resistance estimation and precision therapy methods (Katale et al., 2020).

3. 3. Predicting Drug Resistance Using Computational Models and Machine Learning

Due to the complexity of genetic resistance, machine learning models have become a powerful method for analysing genomic data and can assess a resistant strain with higher precision than mutation detection governed by rules. The prediction of resistance to various anti-TB drugs is enhanced through the use of algorithms like neural networks and support vector machines, which enhances sensitivity and specificity. Such models will be able to identify multidimensional genetic data resistance patterns, including unseen or compound patterns that offer greater scope of prediction and less human error. Such a method is promising to be used clinically and epidemiologically in surveillance and customised interventions (Guyeux et al., 2024; Lam et al., 2021).

A schematic diagram of the epitope-based vaccine development pipeline composed of antigen selection, epitope prediction, the analysis of the population coverage, molecular docking and the in silico validation considering three dimensions is illustrated in Figure 3.

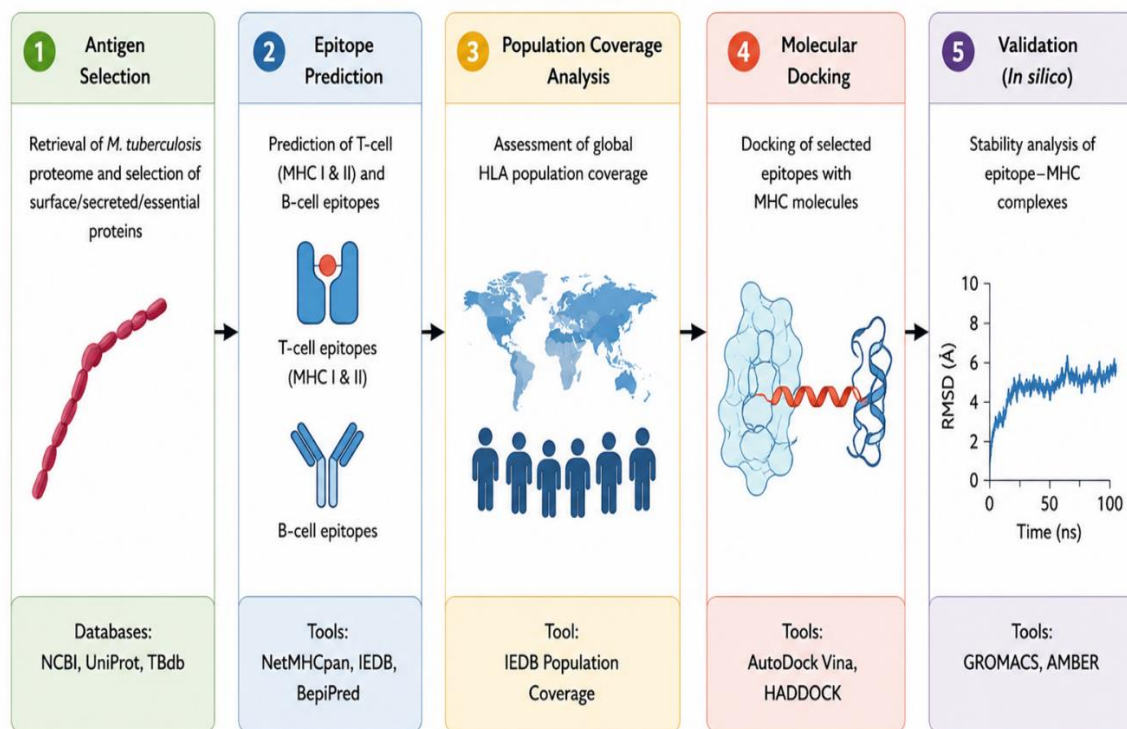


Figure 3. In Silico Pipeline for Epitope-Based Vaccine Design Against *M. tuberculosis*.

4. Bioinformatics and Proteomic Applications to Study *M. tuberculosis*

4. 1. *M. tuberculosis* Proteomics Research

Proteomics plays a very important part in offering vital information about proteins that play roles in bacterial resistance mechanisms and pathogenicity. The methods to study new resistance-associated proteins and proteins that help bacteria to survive within hosts can be identified using such techniques as mass spectrometry. This kind of knowledge can aid in finding drug targets and vaccine contents. In vivo and in vitro proteomic analyses during drug and immune challenges provide a memory of unknown bacterial adaptive strategies and resistance mechanisms (Couvin et al., 2025). Combining proteomics and genomics improves insights in the cellular architecture and functions of bacteria (Alaridah et al., 2019).

4. 2. Molecular Structure of Major Proteins as Targets of the Drugs

X-ray crystallography, among other methods, is a structural study that has led to the elucidation of the 3D structure of key proteins such as diaminopimelate decarboxylase (DAPDC), which is critical for lysine biosynthesis and bacterial survival. Knowledge of structural regions and binding sites contributes to the design of inhibitors of these proteins. Other complexes with toxin-antitoxin complexes, like VapBC, that control growth and persistence are also studied, where bioinformatics tools can be used to identify and target interaction sites for therapeutic intervention ([Guyeux et al., 2021](#); [Hatherell et al., 2016](#)).

4. 3. Protein Structure Prediction and Genetic Modifications via Computational Simulations

Computational simulations model the influence of genetic mutations on the structure and function of proteins together with the sites and strengths of drug binding. They help in the interpretation of resistance mechanisms at a molecular level by predicting dynamic changes because the movement of mutation can also be achieved. The use of molecular dynamics with the help of machine learning allows narrowing down predictions about protein-drug interactions further, thus enabling rational drug design against resistant strains ([Lam et al., 2021](#); [Zhang et al., 2023a](#)).

5. Design of Vaccines against Tuberculosis with the Help of Bioinformatics

5. 1. Contemporary Vaccine Plans and Multiple-Dose Vaccination Plans

Despite more than a hundred years of use, the *Bacillus Calmette-Guerin* (BCG) vaccine still shows low efficacy, especially in cases of pulmonary TB in mature individuals. Bioinformatics in vaccine design provides ample scope to use the whole proteome and pick numerous epitopes to generate broad immune responses in modern vaccine designs. Multi-dose epitope-selected vaccines are designed to generate cellular and humoral immunity, which enhances long-remembered defenses and minimizes evasion by immune bacteria. Vaccines can be supplemented by immune adjuvants which boost their effectiveness ([He et al., 2020](#); [Zhang et al., 2023b](#)).

5. 2. Prediction and Immunological Evaluation with Computational Resources

Scholars employ tools of epitope prediction, molecular docking, and molecular dynamics simulation to determine the parts of peptides that might create an immunologic response. The tools used to predict Major Histocompatibility Complex (MHC) binding assist in the selection of potential peptides. Examples include peptides from the EsxG-EsxH and Rv3587c complexes, which have demonstrated *in silico* potential as vaccines, exhibiting immune activation and blocking bacterial entry into host cells ([Chisompola et al., 2021](#); [Hussien et al., 2022](#)).

5. 3. The Challenges of the Future in TB Vaccine Improvement and Development

Genetic variability of *M. tuberculosis* strains is a problem as far as wide-coverage vaccination is concerned. Although bioinformatics can make the process of searching for vaccine candidates faster and epitope selection easier, extensive clinical testing is required before confirming the safety and efficacy of the vaccine in humans. It is essential to incorporate computational prediction and experimental immunology. A contributing factor to future success is the continued improvement of algorithms to streamline epitope selection and enhance vaccine formulations ([Rosenthal et al., 2017](#)).

6. Role of Biological and Metabolomics Analyses in Tuberculosis Research

6. 1. Advanced Protein and Metabolite Profiling in *M. tuberculosis*

Metabolomics supplements proteomics due to the proficiency of small-molecule bacterial and host metabolites during infection. The approach allows the identification of biomarkers that can be adopted to diagnose TB, distinguish between latent and active infections, and guide treatment. Although the results are good, the complex data of metabolomics require strong bioinformatics to analyse and to validate biomarkers suitable for clinical practice ([Alaridah et al., 2019](#); [Jajou et al., 2019](#)).

6. 2. Tuberculosis and the Microbiome Interactions

Respiratory tract microbiota studies through 16S rDNA-based sequencing have yielded a change in the microbe population of the TB patients, as compared to the healthy members. An increased abundance of genera such as *Cupriavidus* and *Porphyromonas* in the sites of lesions demonstrates potential roles either as cofactors or contributors to the pathogenesis or secondary infections of TB. To study host-pathogen-microbiome interactions, bioinformatics tools come in handy in the analysis of microbial diversity ([A. Dawood & Alnori, 2020](#)). Critically, changes in the respiratory microbiota with TB can affect host immune function and cellular metabolism. Altered microbial metabolites resulting from symbiosis can impact macrophage activation, lipid metabolism, amino acid biosynthesis and inflammatory signaling pathways to establish a metabolic niche that promotes the persistence and progression of *Mycobacterium tuberculosis*. A combination of microbiome data with metabolomics and transcriptomics could offer a greater understanding of the interaction between host and microbe and help to identify new biomarkers and drug targets for TB.

6. 3. Leveraging of Multi-Omics Data to Research on Biological Interactions

Combining multi-omics data, i.e., genomic, proteomic and metabolomic data, makes it possible to take a host-microbe interactome during TB infection and progression into account comprehensively by modelling it. Such complex data are analyzed using cutting-edge computational techniques such as artificial intelligence to unveil biologically relevant models that can be inaccessible by conventional methods. These understandings are utilized in the development of specific preventive and treatment approaches at different stages of the disease ([Kargarpour Kamakoli et al., 2020](#); [Morey-León et al., 2023](#)).

7. Computational Modelling and Viral Surveys *M. tuberculosis* Study

7. 1. GenH Networks and Toxin-Antitoxin Systems

The toxin-antitoxin (TA) systems, including the VapBC system of *M. tuberculosis*, control bacterial growth arrest, adaptation to stress, and dormancy. Bioinformatics and structural analyses show the mechanisms of interaction between toxin and antitoxin moieties, which is crucial to the regulation of survival of the bacteria in an environment that is not accommodating. Computational models of various scenarios of survival provide possible options for biotechnological interventions of disrupting TA systems as antimicrobial measures ([Hatherell et al., 2016](#); [Moco et al., 2022](#)).

7. 2. Functional Prediction of *M. tuberculosis* Proteins

The structural modelling methods are used to identify the active and binding active sites of proteins that are associated with drug resistance, explaining the effects of genetic mutations on molecular activity. The integration between genetic and functional studies leads to rational drug target discovery and is the basis for discovering drug interaction sites, which are important in the development of next-generation anti-TB drugs ([Genestet et al., 2022](#); [Leong et al., 2022](#)).

7. 3. Simulations in the Development of New Drugs

The binding interactions between proteins and drugs, as well as the impact of mutations on binding affinity and stability, are investigated by the means of molecular dynamics simulations. These experiments fulfil the resistance mechanisms and estimate the efficacy of the drugs under study. Computational methods accelerate drug discovery by pre-evaluating molecular interactions before involving them in costly tests in a lab ([Kohl et al., 2018](#); [Lam et al., 2021](#)).

8. Machine Learning and Artificial Intelligence in Diagnosis and Monitoring of TB Infections

8. 1. Machine Learning Model Formulation

Machine learning algorithms increase the sensitivity and speed of identification of drug-resistant *M. tuberculosis* using genomic data. Whereas rigid rule-based tests are rather insensitive in the classification of resistant strains, the models are sensitive in the classification of resistant strains compared to the rule-based test. These models enable predicting resistance to multiple drugs simultaneously. Machine learning is becoming an irreplaceable part of TB surveillance and clinical decision-making at the global level ([Guyeux et al., 2024](#); [Haft et al., 2024](#)).

8. 2. The Use of Computational Tools to Speed Vaccines/Drugs Development

Artificial intelligence (AI) is used to process huge genomic and proteomic data to select interesting drug and vaccine targets. Molecular modelling using AI is used to predict host-pathogen interactions and to assess the work of vaccines on them, which can save development time and cost significantly. In recent years, these technologies have exhibited significant improvements in the identification of proteins that trigger the best immune responses and in the facilitation of successes of TB control programmes ([Tafaj et al., 2020](#); [Zhang et al., 2023a](#)).

8. 3. Hidden Value and Difficulties of Applying AI in TB Healthcare

Implementation of AI in clinical laboratories enhances the speed of data processing and healthcare system reaction. Nevertheless, issues such as regulation, privacy, and reliability come into play. Specific training of staff and modernization of infrastructure are critical measures that could ensure the effective integration of AI into the TB diagnosis and treatment guidelines ([Kargarpour Kamakoli et al., 2020](#); [Morey-León et al., 2023](#)).

9. Future Directions and Research Trends in Bioinformatics for TB

9. 1. Innovations in Genomic Data Collection and Analysis

This changing trend is also characterized by the move to next-generation sequencing (NGS) technology to produce multi-layered genomic and proteomic data. Collaboration has resulted in the merging of datasets to pursue a more comprehensive understanding of bacterial biology and bacterial host interactions. The introduction of new tools incorporating artificial intelligence and cloud computing also allows sophisticated, easy-to-use data analysis and even continuously updated databases of genomes with the most recent results ([Mekonnen et al., 2023](#)).

9. 2. Combination of Bioinformatics and Epidemiological Research/Disease Surveillance

Whole-genome sequencing also enables accurate monitoring of transmission dynamics and the occurrence of mutations in TB. Sophisticated databases and open systems allow data sharing worldwide and collaborative epidemiologic research that helps in a timely response to outbreaks and the conduction of a set of public health programmes ([A. A. Dawood, 2024](#)). The models built on this integration are predictive, which allows managing the spread of diseases and maximizing the quality of healthcare.

9. 3. Technical and Applied Problems of Implementing Bioinformatics Results

However, a high potential is accompanied by obstacles, such as making data sources and methods of data analysis standardised across borders. Infrastructure and skilled workforce do not develop in low-income regions because of scarce resources. To overcome these gaps, policies should work to facilitate sustainable investments, cross-country collaboration, and lifelong learning to build upon and bolster bioinformatics-assisted TB research and clinical practice ([Bakuła, Dziurzyński, et al., 2023](#); [Yin et al., 2023a](#)).

10. Conclusion and Recommendations for Future Research on *M. tuberculosis* and Bioinformatics

10. 1. Summary of Current Bioinformatics Achievements in TB Research

Research on *M. tuberculosis* has undergone a revolution with bioinformatics due to the fact that knowledge about genome complexity, drug resistance mechanisms, diagnosis, and treatment mechanisms has been significantly fast-tracked. Genomics, proteomics, and computational sciences have been integrated to provide new solutions and to improve outcomes in TB control. The developments in the field of sequencing, the analysis of mutation, and the designing of vaccines would be both a major step forward and a bright hope for the populations affected by them around the world ([Couvin et al., 2025](#); [Yin et al., 2023b](#)).

10. 2. The Potential Role of the Emerging Technologies in TB Control

Trends in the future point to the increasing application of AI and machine learning in the creation of fast, sensitive diagnostic methods and in the resolution of intricate genetic patterns of resistance. Such technologies are capable of managing large amounts of data and displaying real-time, actionable insights that can make the decisions of healthcare providers more evidence-based. Avant-garde, data analytics-assisted, collaborative, cross-disciplinary research regimes are likely to facilitate international TB control activities ([Chesov et al., 2022](#); [Guyeux et al., 2024](#)).

10. 3. Recommendations for Research and Health Policy

Investments in bioinformatics infrastructure and technologies through sustainability are very important and required mainly among the high-burden countries. International data sharing and the utilization of open bioinformatics tools should be promoted by health policies so that effective responsive solutions can be developed. Training of clinicians and researchers and continuous development of skills are vital factors that will lead to the success of this multidisciplinary model in TB research and care implementation ([Bakuła, Dziurzyński, et al., 2023](#)).

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

References

- Acharya, B., Acharya, A., Gautam, S., Ghimire, S. P., Mishra, G., Parajuli, N., & Sapkota, B. (2020). Advances in diagnosis of Tuberculosis: An update into molecular diagnosis of *Mycobacterium tuberculosis*. *Molecular Biology Reports*, 47(5), 4065–4075. <https://doi.org/10.1007/s11033-020-05413-7>
- Alaridah, N., Hallbäck, E. T., Tångrot, J., Winqvist, N., Sturegård, E., Florén-Johansson, K., Jönsson, B., Tenland, E., Welinder-Olsson, C., Medstrand, P., Kaijser, B., & Godaly, G. (2019). Transmission dynamics study of tuberculosis isolates with whole genome sequencing in southern Sweden. *Scientific Reports*, 9(1), 4931. <https://doi.org/10.1038/s41598-019-39971-z>
- Bakula, Z., Dziurzyński, M., Decewicz, P., Bakonytė, D., Vasiliauskaitė, L., Nakčerienė, B., Krenke, R., Stakėnas, P., & Jagielski, T. (2023). Spoligotyping of *Mycobacterium tuberculosis* – Comparing in vitro and in silico approaches. *Infection, Genetics and Evolution*, 115, 105508. <https://doi.org/10.1016/j.meegid.2023.105508>
- Bakula, Z., Marczak, M., Bluszcz, A., Proboszcz, M., Kościuch, J., Krenke, R., Stakėnas, P., Mokrousov, I., & Jagielski, T. (2023). Phylogenetic relationships of *Mycobacterium tuberculosis* isolates in Poland: The emergence of Beijing genotype among multidrug-resistant cases. *Frontiers in Cellular and Infection Microbiology*, 13, 1161905. <https://doi.org/10.3389/fcimb.2023.1161905>
- Bellad, R., Nagamoti, M., Sharma, P., & Chauhan, D. S. (2022). Spoligotyping of *Mycobacterium tuberculosis* isolates from Pulmonary Tuberculosis patients from North Karnataka, India. *Tropical Doctor*, 52(3), 386–390. <https://doi.org/10.1177/00494755221080584>
- Bogaerts, B., Delcourt, T., Soetaert, K., Boarbi, S., Ceysens, P.-J., Winand, R., Van Braekel, J., De Keersmaecker, S. C. J., Roosens, N. H. C., Marchal, K., Mathys, V., & Vanneste, K. (2021). A Bioinformatics Whole-Genome Sequencing Workflow for Clinical *Mycobacterium tuberculosis* Complex Isolate Analysis, Validated Using a Reference Collection Extensively Characterized with Conventional Methods and In Silico Approaches. *Journal of Clinical Microbiology*, 59(6), e00202-21. <https://doi.org/10.1128/JCM.00202-21>
- Cancino-Muñoz, I., López, M. G., Torres-Puente, M., Villamayor, L. M., Borrás, R., Borrás-Mañez, M., Bosque, M., Camarena, J. J., Colijn, C., Colomer-Roig, E., Colomina, J., Escribano, I., Esparcia-Rodríguez, O., García-García, F., Gil-Brusola, A., Gimeno, C., Gimeno-Gascón, A., Gomila-Sard, B., Gónzales-Granda, D., ... Comas, I. (2022). Population-based sequencing of *Mycobacterium tuberculosis* reveals how current population dynamics are shaped by past epidemics. *eLife*, 11, e76605. <https://doi.org/10.7554/eLife.76605>
- Chen, J., Chen, L., Zhou, M., Wu, G., Yi, F., Jiang, C., Duan, Q., & Zhou, M. (2022). Transmission of multidrug-resistant tuberculosis within family households by DTM-PCR and MIRU-VNTR genotyping. *BMC Infectious Diseases*, 22(1), 192. <https://doi.org/10.1186/s12879-022-07188-7>
- Chesov, E., Chesov, D., Maurer, F. P., Andres, S., Utpatel, C., Barilar, I., Donica, A., Reimann, M., Niemann, S., Lange, C., Crudu, V., Heyckendorf, J., & Merker, M. (2022). Emergence of bedaquiline resistance in a high tuberculosis burden country. *European Respiratory Journal*, 59(3), 2100621. <https://doi.org/10.1183/13993003.00621-2021>
- Chisompola, N. K., Streicher, E. M., Dippenaar, A., Whitfield, M. G., Tembo, M., Mwanza, S., Warren, R. M., & Sampson, S. L. (2021). Drug resistant tuberculosis cases from the Copperbelt province and Northern regions of Zambia: Genetic diversity, demographic and clinical characteristics. *Tuberculosis*, 130, 102122. <https://doi.org/10.1016/j.tube.2021.102122>
- Couvin, D., Allaguy, A.-S., Ez-zari, A., Jagielski, T., & Rastogi, N. (2025). Molecular typing of *Mycobacterium tuberculosis*: A review of current methods, databases, softwares, and analytical tools. *FEMS Microbiology Reviews*, 49, fuaf017. <https://doi.org/10.1093/femsre/fuaf017>

- Couvin, D., Segretier, W., Stattner, E., & Rastogi, N. (2020). Novel methods included in SpolLineages tool for fast and precise prediction of Mycobacterium tuberculosis complex spoligotype families. *Database*, 2020, baaa108. <https://doi.org/10.1093/database/baaa108>
- Dawood, A. A. (2024). Designing an immuno-epitope candidate vaccine from (Opa, ProA, ProB, RmpM and BamD) proteins against *Neisseria gonorrhoeae* and *Neisseria meningitidis*. *Vacunas (English Edition)*, 25(4), 481–491. <https://doi.org/10.1016/j.vacune.2024.10.009>
- Dawood, A., & Alnori, H. A.-M. (2020). Tunicamycin Anticancer Drug May Reliable to Treat Coronavirus Disease-19. *Open Access Macedonian Journal of Medical Sciences*, 8(T1), 129–133. <https://doi.org/10.3889/oamjms.2020.4954>
- Gautam, S. S., Mac Aogáin, M., Cooley, L. A., Haug, G., Fyfe, J. A., Globan, M., & O'Toole, R. F. (2018). Molecular epidemiology of tuberculosis in Tasmania and genomic characterisation of its first known multi-drug resistant case. *PLOS ONE*, 13(2), e0192351. <https://doi.org/10.1371/journal.pone.0192351>
- Genestet, C., Hodille, E., Bernard, A., Vallée, M., Lina, G., Le Meur, A., Refrégier, G., & Dumitrescu, O. (2022). Consistency of Mycobacterium tuberculosis Complex Spoligotyping between the Membrane-Based Method and In Silico Approach. *Microbiology Spectrum*, 10(3), e00223-22. <https://doi.org/10.1128/spectrum.00223-22>
- Guyeux, C., Senelle, G., Le Meur, A., Supply, P., Gaudin, C., Phelan, J. E., Clark, T. G., Rigouts, L., De Jong, B., Sola, C., & Refrégier, G. (2024). Newly Identified Mycobacterium africanum Lineage 10, Central Africa. *Emerging Infectious Diseases*, 30(3). <https://doi.org/10.3201/eid3003.231466>
- Guyeux, C., Sola, C., Noûs, C., & Refrégier, G. (2021). CRISPRbuilder-TB: “CRISPR-builder for tuberculosis”. Exhaustive reconstruction of the CRISPR locus in mycobacterium tuberculosis complex using SRA. *PLOS Computational Biology*, 17(3), e1008500. <https://doi.org/10.1371/journal.pcbi.1008500>
- Haft, D. H., Badretdin, A., Coulouris, G., DiCuccio, M., Durkin, A. S., Jovenitti, E., Li, W., Mersha, M., O'Neill, K. R., Virothaisakun, J., & Thibaud-Nissen, F. (2024). RefSeq and the prokaryotic genome annotation pipeline in the age of metagenomes. *Nucleic Acids Research*, 52(D1), D762–D769. <https://doi.org/10.1093/nar/gkad988>
- Hatherell, H.-A., Colijn, C., Stagg, H. R., Jackson, C., Winter, J. R., & Abubakar, I. (2016). Interpreting whole genome sequencing for investigating tuberculosis transmission: A systematic review. *BMC Medicine*, 14(1), 21. <https://doi.org/10.1186/s12916-016-0566-x>
- He, G., Li, Y., Chen, X., Chen, J., & Zhang, W. (2020). Prediction of treatment outcomes for multidrug-resistant tuberculosis by whole-genome sequencing. *International Journal of Infectious Diseases*, 96, 68–72. <https://doi.org/10.1016/j.ijid.2020.04.043>
- Hussien, B., Zewude, A., Wondale, B., Hailu, A., & Ameni, G. (2022). Spoligotyping of Clinical Isolates of Mycobacterium tuberculosis Complex Species in the Oromia Region of Ethiopia. *Frontiers in Public Health*, 10, 808626. <https://doi.org/10.3389/fpubh.2022.808626>
- Jajou, R., Kohl, T. A., Walker, T., Norman, A., Cirillo, D. M., Tagliani, E., Niemann, S., De Neeling, A., Lillebaek, T., Anthony, R. M., & Van Soolingen, D. (2019). Towards standardisation: Comparison of five whole genome sequencing (WGS) analysis pipelines for detection of epidemiologically linked tuberculosis cases. *Eurosurveillance*, 24(50). <https://doi.org/10.2807/1560-7917.ES.2019.24.50.1900130>
- Kargarpour Kamakoli, M., Farmanfarmaei, G., Masoumi, M., Khanipour, S., Gharibzadeh, S., Sola, C., Fateh, A., Siadat, S. D., Refregier, G., & Vaziri, F. (2020). Prediction of the hidden genotype of mixed infection strains in Iranian tuberculosis patients. *International Journal of Infectious Diseases*, 95, 22–27. <https://doi.org/10.1016/j.ijid.2020.03.056>
- Katale, B. Z., Mbebele, P. M., Lema, N. A., Campino, S., Mshana, S. E., Rweyemamu, M. M., Phelan, J. E., Keyyu, J. D., Majigo, M., Mbugi, E. V., Dockrell, H. M., Clark, T. G., Matee, M. I., & Mpagama, S. (2020). Whole genome sequencing of

- Mycobacterium tuberculosis isolates and clinical outcomes of patients treated for multidrug-resistant tuberculosis in Tanzania. *BMC Genomics*, 21(1), 174. <https://doi.org/10.1186/s12864-020-6577-1>
- Kohl, T. A., Utpatel, C., Schleusener, V., De Filippo, M. R., Beckert, P., Cirillo, D. M., & Niemann, S. (2018). MTBseq: A comprehensive pipeline for whole genome sequence analysis of Mycobacterium tuberculosis complex isolates. *PeerJ*, 6, e5895. <https://doi.org/10.7717/peerj.5895>
- Lam, C., Martinez, E., Crighton, T., Furlong, C., Donnan, E., Marais, B. J., & Sintchenko, V. (2021). Value of routine whole genome sequencing for Mycobacterium tuberculosis drug resistance detection. *International Journal of Infectious Diseases*, 113, S48–S54. <https://doi.org/10.1016/j.ijid.2021.03.033>
- Leong, K. W. C., Gautam, S. S., Pradhan, M., Singh, Y. I., Kc, R., Rajbhandari, S. K., Ghimire, G. R., Adhikari, K., Shrestha, U., Chaudhary, R., Ghimire, G., Khadka, S., & O'Toole, R. F. (2022). Comparative genomic analyses of multi-drug resistant Mycobacterium tuberculosis from Nepal and other geographical locations. *Genomics*, 114(2), 110278. <https://doi.org/10.1016/j.ygeno.2022.110278>
- Mekonnen, D., Munsha, A., Nibret, E., Adnew, B., Herrera-Leon, S., Amor Aramendia, A., Benito, A., Abascal, E., Jacqueline, C., Aseffa, A., & Herrera-Leon, L. (2023). Comparative whole-genome sequence analysis of Mycobacterium tuberculosis isolated from pulmonary tuberculosis and tuberculous lymphadenitis patients in Northwest Ethiopia. *Frontiers in Microbiology*, 14, 1211267. <https://doi.org/10.3389/fmicb.2023.1211267>
- Moco, V., Cazenave, D., Garnier, M., Pot, M., Marcelino, I., Talarmin, A., Guyomard-Rabenirina, S., Breurec, S., Ferdinand, S., Dereeper, A., Reynaud, Y., & Couvin, D. (2022). getSequenceInfo: A suite of tools allowing to get genome sequence information from public repositories. *BMC Bioinformatics*, 23(1), 268. <https://doi.org/10.1186/s12859-022-04809-5>
- Morey-León, G., Mejía-Ponce, P. M., Granda Pardo, J. C., Muñoz-Mawyin, K., Fernández-Cadena, J. C., García-Moreira, E., Andrade-Molina, D., Licon-Cassani, C., & Berná, L. (2023). A precision overview of genomic resistance screening in Ecuadorian isolates of Mycobacterium tuberculosis using web-based bioinformatics tools. *PLOS ONE*, 18(12), e0294670. <https://doi.org/10.1371/journal.pone.0294670>
- Puustinen, K., Marjamäki, M., Rastogi, N., Sola, C., Filliol, I., Ruutu, P., Holmström, P., Viljanen, M. K., & Soini, H. (2003). Characterization of Finnish Mycobacterium tuberculosis Isolates by Spoligotyping. *Journal of Clinical Microbiology*, 41(4), 1525–1528. <https://doi.org/10.1128/JCM.41.4.1525-1528.2003>
- Rosenthal, A., Gabrielian, A., Engle, E., Hurt, D. E., Alexandru, S., Crudu, V., Sergueev, E., Kirichenko, V., Lapitskii, V., Snezhko, E., Kovalev, V., Astrovko, A., Skrahina, A., Taaffe, J., Harris, M., Long, A., Wollenberg, K., Akhundova, I., Ismayilova, S., ... Tartakovsky, M. (2017). The TB Portals: An Open-Access, Web-Based Platform for Global Drug-Resistant-Tuberculosis Data Sharing and Analysis. *Journal of Clinical Microbiology*, 55(11), 3267–3282. <https://doi.org/10.1128/JCM.01013-17>
- Sola, C., Filliol, I., Gutierrez, M. C., Mokrousov, I., Vincent, V., & Rastogi, N. (2001). Spoligotype Database of Mycobacterium tuberculosis: Biogeographic Distribution of Shared Types and Epidemiologic and Phylogenetic Perspectives. *Emerging Infectious Diseases*, 7(3), 390–396. <https://doi.org/10.3201/eid0703.010304>
- Tafaj, S., Ghariani, A., Trovato, A., Kapisyzi, P., Essalah, L., Mehiri, E., Kasmi, G., Burazeri, G., Slim Saidi, L., & Cirillo, D. M. (2020). Accuracy of the QIAxcel Automated System for MIRU-VNTR Genotyping of Mycobacterium tuberculosis in Two Limited Resource Settings. *Journal of Clinical Medicine*, 9(2), 389. <https://doi.org/10.3390/jcm9020389>
- Yin, C., Mijiti, X., Liu, H., Wang, Q., Cao, B., Anwaierjiang, A., Li, M., Liu, M., Jiang, Y., Xu, M., Wan, K., Zhao, X., Li, G., & Xiao, H. (2023a). Molecular Epidemiology of Clinical Mycobacterium tuberculosis Isolates from Southern Xinjiang, China Using Spoligotyping and 15-Locus MIRU-VNTR Typing. *Infection and Drug Resistance*, Volume 16, 1313–1326. <https://doi.org/10.2147/IDR.S393192>

- Yin, C., Mijiti, X., Liu, H., Wang, Q., Cao, B., Anwaierjiang, A., Li, M., Liu, M., Jiang, Y., Xu, M., Wan, K., Zhao, X., Li, G., & Xiao, H. (2023b). Molecular Epidemiology of Clinical Mycobacterium tuberculosis Isolates from Southern Xinjiang, China Using Spoligotyping and 15-Locus MIRU-VNTR Typing. *Infection and Drug Resistance*, Volume 16, 1313–1326. <https://doi.org/10.2147/IDR.S393192>
- Zhang, X., Martinez, E., Lam, C., Crighton, T., Sim, E., Gall, M., Donnan, E. J., Marais, B. J., & Sintchenko, V. (2023a). Exploring programmatic indicators of tuberculosis control that incorporate routine Mycobacterium tuberculosis sequencing in low incidence settings: A comprehensive (2017–2021) patient cohort analysis. *The Lancet Regional Health - Western Pacific*, 41, 100910. <https://doi.org/10.1016/j.lanwpc.2023.100910>
- Zhang, X., Martinez, E., Lam, C., Crighton, T., Sim, E., Gall, M., Donnan, E. J., Marais, B. J., & Sintchenko, V. (2023b). Exploring programmatic indicators of tuberculosis control that incorporate routine Mycobacterium tuberculosis sequencing in low incidence settings: A comprehensive (2017–2021) patient cohort analysis. *The Lancet Regional Health - Western Pacific*, 41, 100910. <https://doi.org/10.1016/j.lanwpc.2023.100910>



Evaluating Neonatal CPR Diagnostic Accuracy

Safa Abdalla ^{1*}, Manal Taha ², and Hassan Abdelnour ^{3,4} 

¹ Pediatric department, Sultanate of Oman, Sultan Gabos hospital, Dhofar, Oman.

² Pediatric Department, Elsewedy Pediatric Charity Hospital, Khartoum, Sudan. Faculty of Medicine, Al Mughtaribeen University, Khartoum, Sudan.

³ Department of Rehabilitation Sciences. College of Medical Sciences, Northern Border University, Arar, KSA.

⁴ Physiotherapy Department, School of Health Sciences, Ahfad University for Women (AUW), Omdurman, Sudan.



ARTICLE INFO

Received: 05th April 2026
Received in revised form: 19th June 2026
Accepted: 22th June 2026
Published: 15th September 2026

*Corresponding Author:

Safa Abdalla

E-mail :

Safoya762@gmail.com

Citation : Abdalla, S, Taha, M, & Abdelnour, H. (2026). Evaluating Neonatal CPR Diagnostic Accuracy. *Modern Journal of Health and Applied Sciences*, 3(2), 31-41.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Improving clinical outcomes in pediatric care requires the integration of age-appropriate diagnostic and therapeutic interventions. This study aims to evaluate the diagnostic accuracy of CPR among neonates at El-Sewedy Hospital, providing localised evidence to improve the speed and precision of sepsis management in Khartoum. This observational, hospital-based, retrospective cross-sectional study was conducted at the Neonatal Intensive Care Unit of El-Sewedy Pediatric Charity Hospital in Khartoum, Sudan, from January 2022 to January 2023. A convenience sample of 182 neonates (age 28 days and less) with confirmed sepsis was selected, and data regarding demographics, clinical risk factors, and laboratory findings were extracted using a structured form. Data were analysed using SPSS 25.0 with descriptive statistics and bivariate analysis (Chi-square and t-tests), with significance set at a p-value of 0.05 and less. The study secured ethical approval from the Sudan Medical Specialization Board, ensuring data anonymity and confidentiality. The study cohort was predominantly male (57.7%) and preterm (58.2%). The study results showed a 12.1% mortality rate. A significant majority exhibited low birth weight, with very low birth weight (VLBW) being the most common (36.3%). Maternal comorbidities were present in 44% of cases, with urinary tract infections (12.6%) and diabetes (7.6%) being the most frequent. The primary clinical presentations included respiratory distress (69.8%), dysthermia (62.6%), pyrexia (25.2%), and feeding intolerance (20.3%). Other significant findings included hyperbilirubinemia and glycemic instability (both 52.2%). In Khartoum, preterm and low-birth-weight infants face the highest risk of sepsis. Using C-Reactive Protein (CRP) within the Symptoms-Based Score (SBS) React framework provides a fast and reliable diagnostic link to clinical symptoms.

Keywords: Neonatal sepsis, C-reactive protein, Diagnostic accuracy, Blood culture, Neonatal intensive care unit, Biomarkers.

DOI: <https://doi.org/10.70411/MJHAS.3.2.2026378>

1. Introduction

Neonatal sepsis continues to be a formidable clinical challenge, acting as a primary driver of neonatal morbidity and mortality in Neonatal Intensive Care Units (NICUs) globally. It is defined as a systemic inflammatory response syndrome occurring within the first 28 days of life, triggered by suspected or proven infection (Folgori & Bielicki, 2019). Despite advancements in neonatal care, recent global estimates indicate that sepsis remains responsible for a significant proportion of the 2.3 million neonatal deaths occurring annually, with a disproportionate burden falling on low- and middle-income countries (Satti et al., 2025; World Health Organization, 2024). In regions such as sub-Saharan Africa, including Sudan, the prevalence is exacerbated by high rates of home births and limited access to immediate specialized care (Seale et al., 2013, 2014).

The clinical diagnosis of neonatal sepsis is notoriously complex because the initial signs, such as temperature instability, poor feeding, and lethargy, are subtle and highly non-specific, frequently overlapping with other neonatal conditions like respiratory distress or prematurity-related complications (Coetzee et al., 2017; Kariniotaki et al., 2024). Because the disease progresses with devastating speed, clinicians are often compelled to initiate empirical antibiotic therapy before laboratory confirmation. However, this "safety-first" approach has led to the excessive use of broad-spectrum antibiotics, which is a major driver of the emerging global crisis of multidrug-resistant organisms (Rallis et al., 2023).

Blood culture remains the microbiological reference standard for confirming neonatal sepsis. However, its usefulness for early clinical decision-making is limited by the time required to obtain definitive results, typically 36–48 hours, and by imperfect sensitivity. Diagnostic yield can be reduced when inadequate blood volumes are collected, particularly in neonates with low-level or intermittent bacteraemia; maternal intrapartum antimicrobial exposure may also complicate organism recovery (Celik et al., 2022; Iroh Tam & Bendel, 2017). Consequently, many neonates receive empirical treatment for suspected or "culture-negative" sepsis despite sterile blood cultures. This diagnostic uncertainty creates a need for rapid, accurate, affordable, and clinically validated biomarkers to support early treatment decisions and antimicrobial stewardship (Klingenberg et al., 2018; Sturrock et al., 2023).

Among these markers, C-Reactive Protein (CRP) remains the most widely utilised acute-phase reactant in the NICU setting. CRP is synthesised by the liver in response to inflammatory cytokines like IL-6 during infection (Emokpae et al., 2025). While CRP is highly valued for its accessibility and low cost—crucial factors in resource-constrained hospitals its interpretation requires precision. CRP levels may rise due to non-infectious stressors, necessitating a clear understanding of their diagnostic accuracy, specifically their sensitivity and specificity, within specific clinical contexts (Beltempo et al., 2018; Gilfillan & Bhandari, 2019). A positive or elevated CRP means that the body is fighting infection or inflammation. The SBSReact (or SBSReaction) framework is a specialised clinical protocol designed for the rapid diagnosis and management of neonatal sepsis (blood infections in newborns), particularly in resource-limited healthcare settings.

At El-Sewedy Pediatric Charity Hospital in Khartoum, Sudan, the neonatal intensive care unit serves neonates who may be at high risk of severe infection and adverse outcomes. In this setting, timely identification of neonatal sepsis is essential because delays in diagnosis and treatment can be life-threatening, whereas unnecessary empirical antibiotic exposure may contribute to antimicrobial resistance (Brima et al., 2021; Murless-Collins et al., 2023). Establishing a locally validated CRP threshold against blood-culture results could support more timely clinical decision-making, improve diagnostic precision, and strengthen antimicrobial stewardship (Murless-Collins et al., 2023; Sarafidis, 2023). This study therefore aims to evaluate the diagnostic accuracy of serum CRP for neonatal sepsis among neonates admitted to the NICU at El-Sewedy Pediatric Charity Hospital, using blood culture as the microbiological reference standard.

2. Methodology

This research utilizes a retrospective observational cross-sectional, hospital-based design to investigate the clinical profile of neonatal sepsis at the Neonatal Intensive Care Unit (NICU) of El-Sewedy Pediatric Charity Hospital in Khartoum, Sudan. Established in 2006, this specialized facility serves as a critical regional hub for neonatal care. The NICU features four specialised areas with a capacity of 15 neonates and is equipped with advanced life-support technology, including 12 mechanical ventilators, 20 CPAP machines, 40 incubators, and 20 phototherapy units. Diagnostic capabilities are supported by on-site arterial blood gas (ABG) and electrolyte analysis, portable X-ray, and ultrasound services. Additionally, the unit facilitates advanced interventions such as umbilical venous catheters (UVCs) and peripherally inserted central catheters (PICCs).

The study population comprised neonates aged 28 days or less who were admitted between January 2022 and January 2023. Inclusion required a confirmed diagnosis of neonatal sepsis based on clinical presentation and dual laboratory confirmation via C-reactive protein (CRP) testing and blood culture results. Only patients with complete, high-quality medical records were enrolled, while those failing to meet these diagnostic or administrative benchmarks were excluded.

Given the known population of 334 septic neonates during the study period, the sample size was determined using the formula $n = N / (1 + N(e)^2)$. With a 0.05 margin of error, a sample of 182 participants was established via convenience sampling. Data were systematically extracted from medical records using a structured, close-ended form designed to capture demographic, clinical characteristics, and longitudinal laboratory data.

Statistical analysis was performed using SPSS version 25.0. Descriptive statistics summarised baseline characteristics, using frequencies and percentages for categorical variables and means with standard deviations for continuous variables. To identify significant associations, the Chi-square test or Fisher's exact test was applied to categorical data, while independent t-tests or Mann-Whitney U tests were used for continuous data. Statistical significance was set at $p < 0.05$.

Ethical integrity was maintained according to the Declaration of Helsinki, with formal approval from the hospital's Research Ethics Committee and the Ministry of Health. A waiver of informed consent was granted for this retrospective study, though strict confidentiality was maintained through data anonymisation and password-protected electronic records.

3. Results

Table 1. Demographical data of the study group (n = 182).

		Frequency	Percent
Gender	Male	105	57.7
	Female	77	42.3
Number	Single	143	78.6
	Twin	32	17.6
	Triple	2	1.1
	Quadruplet	5	2.7
Gestational age	Preterm	106	58.2
	Term	76	41.8
Birth Weight	Macrosomia	4	2.2
	Normal	36	19.8
	Low birth weight	47	25.8
	Very low birth weight	66	36.3
	Extremely low birth weight	29	15.9

Table 1, delineates the demographic and clinical characteristics of the study cohort. The sample exhibited a male predominance (57.7% vs. 42.3%), with singleton gestations constituting the majority of births (78.6%), followed by twin pregnancies (17.6%). In terms of clinical parameters, preterm deliveries (58.2%) were more prevalent than term births (41.8%). Neonatal birth weight distribution indicated significant vulnerability; very low birth weight (VLBW) was the most frequent classification (36.3%), followed by low birth weight (LBW; 25.8%) and extremely low birth weight (ELBW; 15.9%). Conversely, normal birth weight and macrosomia represented only 19.8% and 2.2% of the population, respectively.

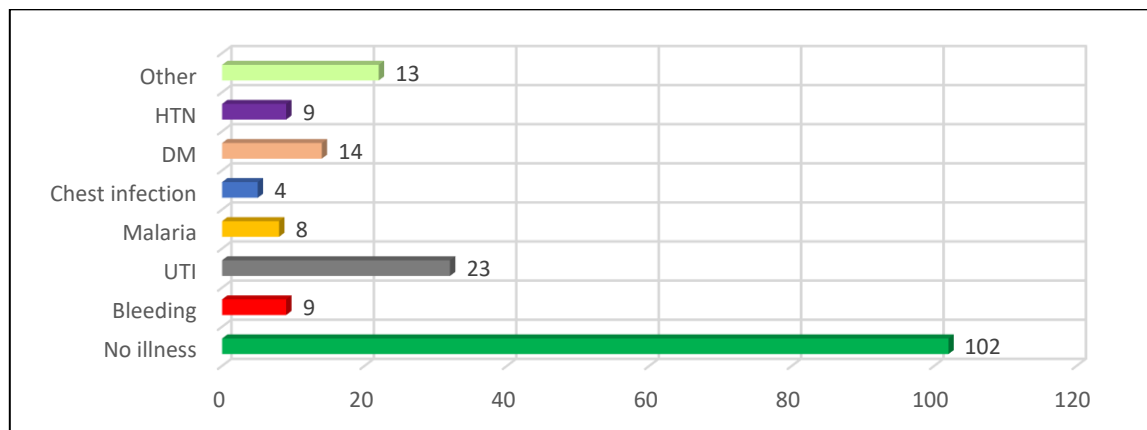


Figure 1. The distribution of the study population according to the mothers' comorbidities (n = 182).

Figure 1 illustrates the distribution of maternal comorbidities within the study population. More than half of the mothers (n=102, 56%) had no medical illness. Among those with documented pathologies, urinary tract infection (UTI) was the most prevalent (n = 23, 12.6%), followed by diabetes mellitus (n = 14, 7.6%), hypertension (n = 9, 4.9%), and antepartum hemorrhage (n = 9, 4.9%). Other recorded conditions included malaria (n = 8, 4.3%) and respiratory tract infections (n = 4, 2.1%).

Table 2 details the frequency of clinical manifestations and signs of neonatal sepsis. The most common presenting symptoms were pyrexia (n = 46, 25.2%), feeding intolerance (n = 37, 20.3%), and lethargy (n = 28, 15.38%). Regarding clinical signs, respiratory distress specifically tachypnea was the most frequent finding (n = 127, 69.78%), followed by dysthermia (n=114, 62.64%). Other significant observations included hyperbilirubinemia (n = 95, 52.20%), glycemic instability (n=95, 52.20%), irritability (n=92, 50.55%), and compromised peripheral perfusion (n=88, 48.35%).

Table 2. The clinical features of the study population (n=182).

		Frequency	Percent
Symptoms	Fever	46	25.2%
	Refuse of feeding	37	20.3%
	Lethargy	28	15.38%
	Vomiting	17	9.3%
	Weak cry	10	5.4%
	Others	44	24.2%
Clinical signs	Thermal instability	114	62.64%
	Irritability	92	50.55%
	Tachycardia	75	41.21%
	Oliguria	19	10.44%

Poor feeding	82	45.05%
Tachypnea	127	69.78%
Poor skin perfusion	88	48.35%
Convulsion	22	12.09%
Jaundice	95	52.20%
Abnormal glucose level	95	52.20%
Abdominal distension	71	39.01%

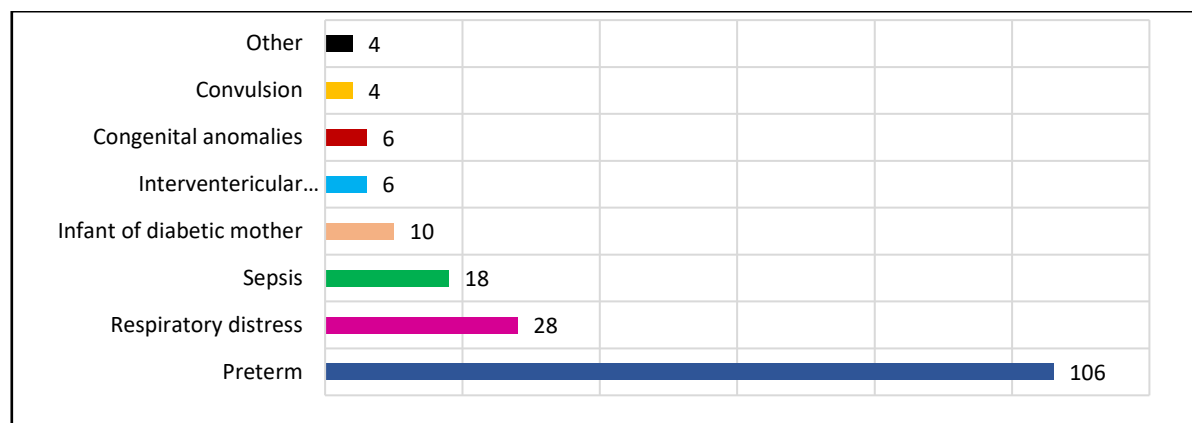


Figure 2. The frequencies of indications for admission for the Neonatal ICU (n=182).

Figure 2 outlines the primary indications for admission among the study subjects. The most frequent cause was preterm birth, accounting for 106 patients (58.2%). This was followed by respiratory distress (n = 28, 15.3%) and neonatal sepsis (n = 18, 9.9%). Less frequent indications included infants of diabetic mothers (IDM) (n = 10, 5.4%), intraventricular hemorrhage (IVH) (n = 6, 3.2%), and congenital anomalies (n = 6, 3.2%), while seizures and other miscellaneous conditions each accounted for 2.1% of admissions.

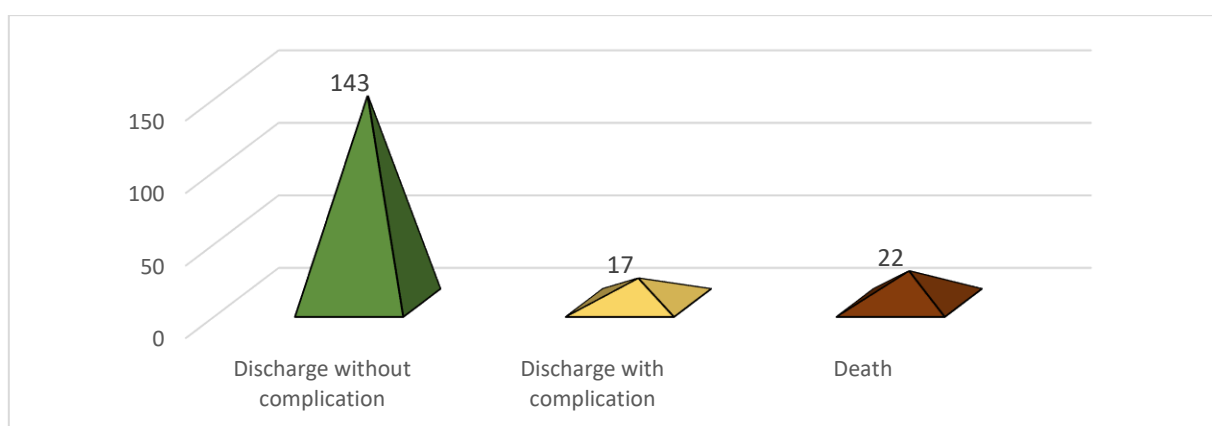


Figure 3. The distribution of the study group according to the outcomes of NICU admission (n = 182).

Figure 3 delineates the clinical outcomes of patients admitted to the Neonatal Intensive Care Unit (NICU). The majority of the cohort (n = 143, 78.6%) was successfully discharged in stable condition. Conversely, morbidity was observed in 17 patients (9.3%), who developed complications, while the mortality rate accounted for 12.1% of the cases (n = 22).

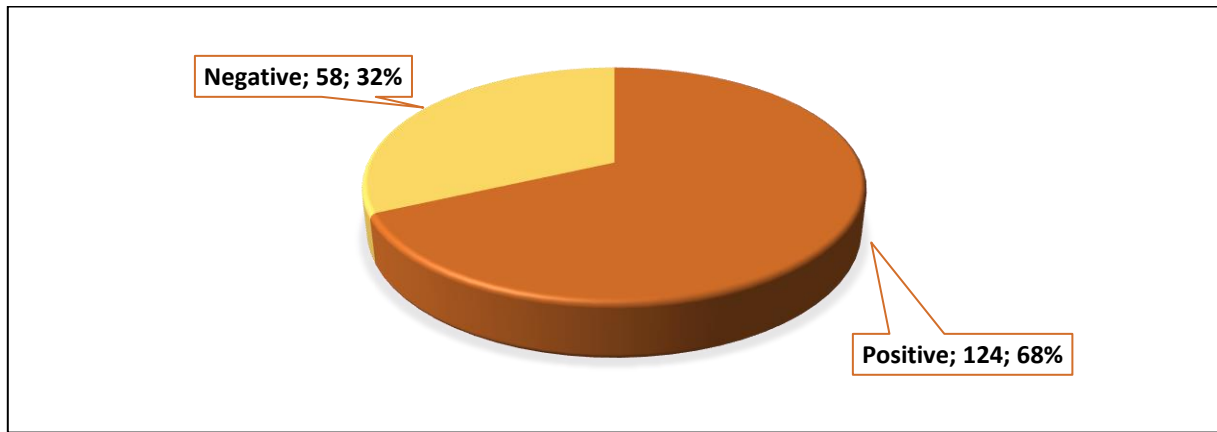


Figure 4. The distribution of the study population according to the results of the Blood culture (n = 182).

Figure 4, illustrates the results of microbiological blood cultures. Culture-proven sepsis was identified in 124 cases (68.1%), while 58 cases (31.9%) yielded negative results, indicating no bacterial growth.

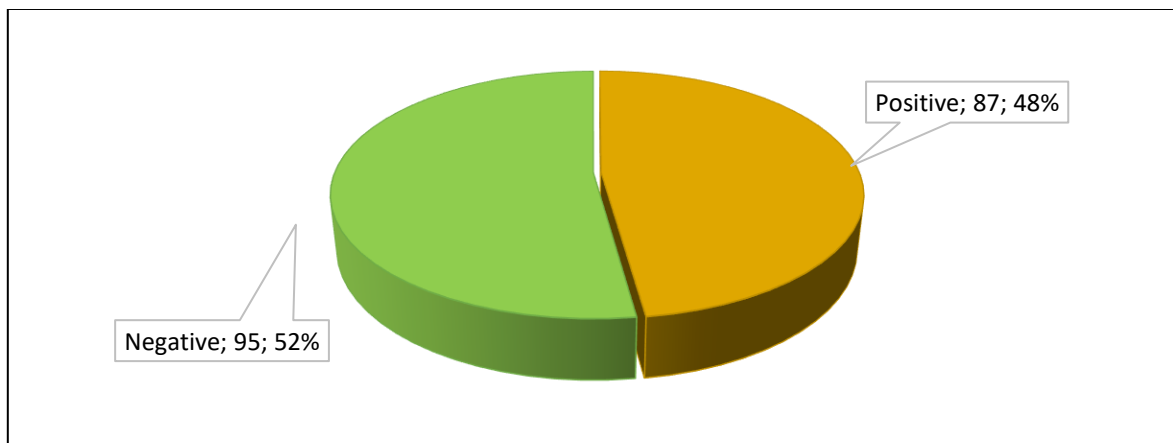


Figure 5. The distribution of the study population according to the results of the CRP (n = 182).

Figure 5, illustrates the distribution of C-reactive protein (CRP) results within the study population, with CRP positivity observed in 48% of cases (n = 87) and negative results in 52% (n = 95).

Table 3, evaluates the statistical correlation between CRP reactivity and various demographic and clinical variables. No statistically significant associations were identified between CRP results and neonatal gender (p = 0.08) or mode of delivery (p = 0.098). However, a highly significant correlation was observed between CRP positivity and both preterm birth (p < 0.0001) and premature rupture of membranes (PROM) (p = 0.008).

Table 3. The correlation between the results of CRP with the gender of neonate, Mode of delivery, gestational age and PROM.

		CRP		Total	P-value
		Positive	Negative		
Gender	Male	56	49	105	0.08
	Female	31	46	77	
NVD		37	52	89	0.098

Mode of delivery	E C/S	8	12	20	
	EM C/S	42	31	73	
Gestational age	Term	9	67	76	< 0.0001*
	Preterm	78	28	106	
PROM	< 18 hours	19	38	57	0.008*
	>18 hours	68	57	125	

*Significant correlation

4. Discussion

The findings provide a descriptive overview of the demographic and diagnostic profile of neonates evaluated for sepsis at El-Sewedy Pediatric Charity Hospital. Male neonates constituted 57.7% of the study cohort, indicating a modest male predominance. This observation is consistent with previous literature reporting greater susceptibility of male neonates to adverse outcomes and several forms of neonatal infection, including sepsis (Kelly et al., 2023; Özmeral Odabaşı, 2020). Biological explanations for this pattern remain multifactorial. The X chromosome contains numerous genes involved in innate immune signaling, and the XX genotype may provide females with immunological advantages through X-linked gene expression and cellular mosaicism. In addition, sex hormones may influence neonatal immune-cell function and inflammatory responses (McGovern et al., 2024). Therefore, the male predominance observed in the present cohort may reflect recognized sex-related differences in neonatal immune vulnerability; however, it should be interpreted alongside other determinants of sepsis risk, including prematurity, birth weight, delivery-related exposures, and local patterns of care.

A critical observation in this cohort was the high prevalence of preterm births and the alarming proportion of neonates classified as very low birth weight (VLBW) (36.3%), low birth weight (LBW) (25.8%), and extremely low birth weight (ELBW) (15.9%), together representing 78% of the sample. These physical vulnerabilities are primary drivers of sepsis, as preterm infants possess an immature skin barrier and a deficiency in IgG antibodies (Lekić et al., 2019). In resource-constrained environments like Khartoum, the management of these high-risk neonates is further complicated by the necessity of invasive procedures, such as umbilical venous catheters (UVCs) (Rees et al., 2023). While life-saving, these interventions serve as potential portals for nosocomial pathogens, especially where environmental colonisation is a constant threat (Popescu et al., 2020). The synergy between prematurity and invasive care creates a "perfect storm" for late-onset sepsis in overcrowded NICUs (Tumuhameye et al., 2020).

The diagnostic utility of C-Reactive Protein (CRP) remains a focal point of clinical debate (Beltempo et al., 2018). In this study, CRP served as a pivotal rapid biomarker. While the "gold standard" blood culture is essential, its application in Sudan is hampered by long incubation periods and high false-negative rates (Rees et al., 2023). These false-negative results are often due to low-volume blood sampling or maternal intrapartum antibiotic use, which suppresses bacterial growth in vitro (Sarkar et al., 2014). CRP synthesis by the liver in response to IL-6 provides a measurable indicator of systemic inflammation (Emokpae et al., 2025). Our findings suggest that within the SBSReact framework, CRP serves as an effective diagnostic adjunct (Liberati et al., 2025). When serial CRP levels remain low, clinicians can consider the early cessation of empirical antibiotics, thereby supporting antimicrobial stewardship (Gkentzi & Dimitriou, 2019). This is crucial in reducing the selection pressure for multidrug-resistant organisms, which represent a growing crisis in sub-Saharan Africa.

Furthermore, CRP interpretation must be contextualized within the neonatal transition period (Eichberger et al., 2022). CRP levels can naturally fluctuate during the first 48 hours due to non-infectious stressors like fetal distress or meconium aspiration (Hofer et al., 2012). Therefore, integrating CRP findings within a specialized framework is vital to avoid overdiagnosis (Cosovanu et al., 2026). The high rate of multiple gestations in this study (over 21%) also suggests that obstetric

factors play a significant role in the local sepsis burden (Seale et al., 2013). This highlights the need for integrated maternal-neonatal health strategies, as proposed by international guidelines (World Health Organization, 2024).

Socio-economic instability in Sudan during the study period (2022-2023) directly impacted medical supply chains and laboratory efficiency (Ahmed et al., 2025; Mohammed et al., 2023). In such a landscape, the SBSReact framework provides a structured pathway to maximize biochemical data utility (Wong et al., 2018). By utilizing CRP as a high-sensitivity screening tool, NICUs can better allocate limited resources to neonates with the highest likelihood of confirmed sepsis (De Rose et al., 2024). This strategy not only improves individual patient outcomes but also enhances overall hospital efficiency by reducing unnecessary hospitalizations among neonates with non-infectious inflammatory responses (Chevalier et al., 2025). Ultimately, the results underscore that a high index of suspicion combined with rapid biomarkers is essential for survival in high-burden settings (Fleischmann-Struzek et al., 2018).

5. Conclusion

This study concludes that neonatal sepsis disproportionately affects preterm and low-birth-weight neonates in Khartoum. C-reactive protein (CRP), when integrated into the SBSReact framework, serves as a vital rapid diagnostic tool that correlates effectively with clinical presentation. While blood cultures remain the gold standard, the prompt use of CRP facilitates timely intervention and supports antimicrobial stewardship in resource-limited settings.

6. Limitations

This retrospective, single-center study, limited by potential selection bias from convenience sampling, may not reflect broader, multi-institutional clinical patterns. Future research should prioritise prospective, multi-center trials incorporating additional, advanced inflammatory biomarkers to enhance diagnostic precision.

Conflict of Interest

The authors declare no conflicts of interest, financial or personal, that could have influenced the work presented in this study.

Data Availability

This research did not receive any specific funding from public, commercial, or non-profit organisations.

Acknowledgments

The authors express their gratitude to the NICU of El-Sewedy Pediatric Charity Hospital in Khartoum, Sudan, for providing the necessary facilities and support to carry out this study.

References

- Ahmed, I., Mergany, E., Alfadil, S., Abdelnour, H., Bleedy, N., & Almalty, A. (2025). Awareness and Experience of Physiotherapy Role in Urinary Incontinence. *Saudi Journal of Nursing and Health Care*, 8, 301–307. <https://doi.org/10.36348/sjnhc.2025.v08i12.003>
- Beltempo, M., Viel-Thériault, I., Thibeault, R., Julien, A.-S., & Piedboeuf, B. (2018). C-reactive protein for late-onset sepsis diagnosis in very low birth weight infants. *BMC Pediatrics*, 18(1), 16. <https://doi.org/10.1186/s12887-018-1002-5>
- Braima, O., Ali, M., & Abdulla, E. (2021). Bacteriological profile and antibiotic resistance in newborn infants with possible community-acquired neonatal sepsis in Khartoum State, Sudan. *Sudanese Journal of Paediatrics*, 13–22. <https://doi.org/10.24911/SJP.106-1601909519>
- Celik, I. H., Hanna, M., Canpolat, F. E., & Mohan Pammi. (2022). Diagnosis of neonatal sepsis: The past, present and future. *Pediatric Research*, 91(2), 337–350. <https://doi.org/10.1038/s41390-021-01696-z>
- Chevalier, J. M., Hansen, M. A., Grantz, K. H., Gleeson, B., Blumel, B., Chuchu, V., Khan, S., Sigwebela, N., Chimhini, G., Fitzgerald, F., Ferreyra, C., & Nichols, B. E. (2025). Potential Health and Cost Impacts of a Point-of-Care Test for Neonatal Sepsis and Possible Serious Bacterial Infections in Infants: A Modeling Analysis in Two Settings. *Open Forum Infectious Diseases*, 12(11), ofaf652. <https://doi.org/10.1093/ofid/ofaf652>
- Coetzee, M., Mbowane, N., & De Witt, T. (2017). Neonatal sepsis: Highlighting the principles of diagnosis and management. *South African Journal of Child Health*, 11(2), 99. <https://doi.org/10.7196/SAJCH.2017.v11i2.1244>
- Cosovanu, E. T., Cosovanu, E. O., Ionescu, S. G., Asaftei, A., Damian, C., Asaftei-Titianu, G. S., Donos, M. A., Petroaie, A. D., Ioniuc, I. K., Petris, O. R., Coman, E. A., Iancu, L. S., Caruntu, I. D., & Ursu, R. G. (2026). Diagnosing Neonatal Sepsis: A Comprehensive Review of Conventional Culture, Molecular Methods, and Host-Response Biomarkers. *Acta Microbiologica Hellenica*, 71(3), 28. <https://doi.org/10.3390/amh71030028>
- De Rose, D. U., Ronchetti, M. P., Martini, L., Rechichi, J., Iannetta, M., Dotta, A., & Auriti, C. (2024). Diagnosis and Management of Neonatal Bacterial Sepsis: Current Challenges and Future Perspectives. *Tropical Medicine and Infectious Disease*, 9(9), 199. <https://doi.org/10.3390/tropicalmed9090199>
- Eichberger, J., Resch, E., & Resch, B. (2022). Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. *Frontiers in Pediatrics*, 10, 840288. <https://doi.org/10.3389/fped.2022.840288>
- Emokpae, U. O., Emokpae, A., & Olajuyigbe, O. O. (2025). Evaluation of Procalcitonin and C-Reactive Protein as an Early Marker for the Diagnosis of Neonatal Sepsis. *Journal of Advances in Microbiology*, 25(10), 60–67. <https://doi.org/10.9734/jamb/2025/v25i101004>
- Fleischmann-Struzek, C., Goldfarb, D. M., Schlattmann, P., Schlapbach, L. J., Reinhart, K., & Kissoon, N. (2018). The global burden of paediatric and neonatal sepsis: A systematic review. *The Lancet Respiratory Medicine*, 6(3), 223–230. [https://doi.org/10.1016/S2213-2600\(18\)30063-8](https://doi.org/10.1016/S2213-2600(18)30063-8)
- Folgori, L., & Bielicki, J. (2019). Future Challenges in Pediatric and Neonatal Sepsis: Emerging Pathogens and Antimicrobial Resistance. *Journal of Pediatric Intensive Care*, 08(01), 017–024. <https://doi.org/10.1055/s-0038-1677535>
- Gilfillan, M., & Bhandari, V. (2019). Neonatal sepsis biomarkers: Where are we now? *Research and Reports in Neonatology*, Volume 9, 9–20. <https://doi.org/10.2147/RRN.S163082>
- Gkentzi, D., & Dimitriou, G. (2019). Antimicrobial Stewardship in the Neonatal Intensive Care Unit: An Update. *Current Pediatric Reviews*, 15(1), 47–52. <https://doi.org/10.2174/1573396315666190118101953>
- Hofer, N., Zacharias, E., Müller, W., & Resch, B. (2012). An Update on the Use of C-Reactive Protein in Early-Onset Neonatal Sepsis: Current Insights and New Tasks. *Neonatology*, 102(1), 25–36. <https://doi.org/10.1159/000336629>
- Iroh Tam, P.-Y., & Bendel, C. M. (2017). Diagnostics for neonatal sepsis: Current approaches and future directions. *Pediatric Research*, 82(4), 574–583. <https://doi.org/10.1038/pr.2017.134>

- Kariniotaki, C., Thomou, C., Gkentzi, D., Panteris, E., Dimitriou, G., & Hatzidaki, E. (2024). Neonatal Sepsis: A Comprehensive Review. *Antibiotics*, 14(1), 6. <https://doi.org/10.3390/antibiotics14010006>
- Kelly, L. A., Branagan, A., Semova, G., & Molloy, E. J. (2023). Sex differences in neonatal brain injury and inflammation. *Frontiers in Immunology*, 14, 1243364. <https://doi.org/10.3389/fimmu.2023.1243364>
- Klingenberg, C., Kornelisse, R. F., Buonocore, G., Maier, R. F., & Stocker, M. (2018). Culture-Negative Early-Onset Neonatal Sepsis—At the Crossroad Between Efficient Sepsis Care and Antimicrobial Stewardship. *Frontiers in Pediatrics*, 6, 285. <https://doi.org/10.3389/fped.2018.00285>
- Lekić, E., Babović, S., Vukićević, J., Nešović, M., & Dragaš, L. (2019). Early-onset neonatal sepsis and risk factors in the preterm infants. *Perinat J*, 27, 143–149.
- Liberati, C., Brigadoi, G., Barbieri, E., Giaquinto, C., & Donà, D. (2025). Antimicrobial Stewardship Programs in Pediatric Intensive Care Units: A Systematic Scoping Review. *Antibiotics*, 14(2), 130. <https://doi.org/10.3390/antibiotics14020130>
- McGovern, M., Kelly, L., Finnegan, R., McGrath, R., Kelleher, J., El-Khuffash, A., Murphy, J., Greene, C. M., & Molloy, E. J. (2024). Gender and sex hormone effects on neonatal innate immune function. *The Journal of Maternal-Fetal & Neonatal Medicine*, 37(1), 2334850. <https://doi.org/10.1080/14767058.2024.2334850>
- Mohammed, F. E. A., Viva, M. I. F., Awadalla, W. A. G., Elmahi, O. K. O., Wainstock, D., & Patil, P. (2023). Defending the right to health during Sudan’s civil war. *The Lancet Global Health*, 11(9), e1327–e1328. [https://doi.org/10.1016/S2214-109X\(23\)00300-5](https://doi.org/10.1016/S2214-109X(23)00300-5)
- Murless-Collins, S., Kawaza, K., Salim, N., Molyneux, E. M., Chiume, M., Aluvaala, J., Macharia, W. M., Ezeaka, V. C., Odedere, O., Shamba, D., Tillya, R., Penzias, R. E., Ezenwa, B. N., Ohuma, E. O., Cross, J. H., Lawn, J. E., the NEST360 Infection Group, Bokea, H., Bohne, C., ... Palamounkian, K. (2023). Blood culture versus antibiotic use for neonatal inpatients in 61 hospitals implementing with the NEST360 Alliance in Kenya, Malawi, Nigeria, and Tanzania: A cross-sectional study. *BMC Pediatrics*, 23(S2), 568. <https://doi.org/10.1186/s12887-023-04343-0>
- Özmeral Odabaşı, I. (2020). Neonatal Sepsis. *SiSli Etfal Hastanesi Tip Bulteni / The Medical Bulletin of Sisli Hospital*. <https://doi.org/10.14744/SEMB.2020.00236>
- Popescu, C. R., Cavanagh, M. M. M., Tembo, B., Chiume, M., Lufesi, N., Goldfarb, D. M., Kissoon, N., & Lavoie, P. M. (2020). Neonatal sepsis in low-income countries: Epidemiology, diagnosis and prevention. *Expert Review of Anti-Infective Therapy*, 18(5), 443–452. <https://doi.org/10.1080/14787210.2020.1732818>
- Rallis, D., Giapros, V., Serbis, A., Kosmeri, C., & Baltogianni, M. (2023). Fighting Antimicrobial Resistance in Neonatal Intensive Care Units: Rational Use of Antibiotics in Neonatal Sepsis. *Antibiotics*, 12(3), 508. <https://doi.org/10.3390/antibiotics12030508>
- Rees, C. A., Lim, J., Westbrook, A. L., El Helou, R., Schmid, A., Rubin-Smith, J., Shreeve, K., Rotman, C., Govindapillai, S., Dorney, K., & Niescierenko, M. (2023). Systematic review and meta-analysis of the diagnostic value of four biomarkers in detecting neonatal sepsis in low- and middle-income countries. *BMJ Paediatrics Open*, 7(1), e001627. <https://doi.org/10.1136/bmjpo-2022-001627>
- Sarafidis, K. (2023). Special Issue “Recent Advances in Neonatal Sepsis”. *Journal of Clinical Medicine*, 12(4), 1385. <https://doi.org/10.3390/jcm12041385>
- Sarkar, S., Bhagat, I., Bhatt-Mehta, V., & Sarkar, S. (2014). Does Maternal Intrapartum Antibiotic Treatment Prolong the Incubation Time Required for Blood Cultures to Become Positive for Infants with Early-Onset Sepsis? *American Journal of Perinatology*, 32(04), 357–362. <https://doi.org/10.1055/s-0034-1387933>
- Satti, L., Abdelnour, H., Yousif, B., Mustafa, O., & Mohammed, R. (2025). Text neck syndrome among students at the National University in Sudan. *World J Adv Res Rev*, 28(3), 1501–1507.

- Seale, A. C., Blencowe, H., Manu, A. A., Nair, H., Bahl, R., Qazi, S. A., Zaidi, A. K., Berkley, J. A., Cousens, S. N., & Lawn, J. E. (2014). Estimates of possible severe bacterial infection in neonates in sub-Saharan Africa, south Asia, and Latin America for 2012: A systematic review and meta-analysis. *The Lancet Infectious Diseases*, 14(8), 731–741. [https://doi.org/10.1016/S1473-3099\(14\)70804-7](https://doi.org/10.1016/S1473-3099(14)70804-7)
- Seale, A. C., Blencowe, H., Zaidi, A., Ganatra, H., Syed, S., Engmann, C., Newton, C. R., Vergnano, S., Stoll, B. J., Cousens, S. N., & Lawn, J. E. (2013). Neonatal severe bacterial infection impairment estimates in South Asia, sub-Saharan Africa, and Latin America for 2010. *Pediatric Research*, 74(S1), 73–85. <https://doi.org/10.1038/pr.2013.207>
- Sturrock, S., Sadoo, S., Nanyunja, C., & Le Doare, K. (2023). Improving the Treatment of Neonatal Sepsis in Resource-Limited Settings: Gaps and Recommendations. *Research and Reports in Tropical Medicine*, Volume 14, 121–134. <https://doi.org/10.2147/RRTM.S410785>
- Tumuhameye, J., Sommerfelt, H., Bwanga, F., Ndeezi, G., Mukunya, D., Napyo, A., Nankabirwa, V., & Tumwine, J. K. (2020). Neonatal sepsis at Mulago national referral hospital in Uganda: Etiology, antimicrobial resistance, associated factors and case fatality risk. *PLOS ONE*, 15(8), e0237085. <https://doi.org/10.1371/journal.pone.0237085>
- Wong, D. M., Ruby, R. E., Dembek, K. A., Barr, B. S., Reuss, S. M., Magdesian, K. G., Olsen, E., Burns, T., Slovis, N. M., & Wilkins, P. A. (2018). Evaluation of updated sepsis scoring systems and systemic inflammatory response syndrome criteria and their association with sepsis in equine neonates. *Journal of Veterinary Internal Medicine*, 32(3), 1185–1193. <https://doi.org/10.1111/jvim.15087>
- World Health Organization. (2024). Networks of care for maternal and newborn health: Implementation guidance. World Health Organization.



Cytokine-Mediated Immune Responses in *Mycobacterium tuberculosis* Infections: Mechanisms, Regulation, and Therapeutic Implications

Ali Adel Dawood ^{1*}

¹ Department of Anatomy, Al-Batool College of Medicine, University of Mosul.

ARTICLE INFO

Received: 18th May 2026
Received in revised form: 20th June 2026
Accepted: 23th June 2026
Published: 15th September 2026

*Corresponding Author:

Ali Adel Dawood
E-mail :
aad@uomosul.edu.iq

Citation: Dawood, A, A (2026). Cytokine-Mediated Immune Responses in *Mycobacterium tuberculosis* Infections: Mechanisms, Regulation, and Therapeutic Implications. *Modern Journal of Health and Applied Sciences*, 3(2), 42-60.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Cytokines are key players in the coordination of immune responses against bacterial pathogens, and their dysregulation leads to immunopathology and normal protective immunity. This review article analyses the elaborate cytokine interactions present in *Mycobacterium tuberculosis* infections, which is one of the major bacterial pathogens posing health challenges in the world. We study the various roles played by pro-inflammatory cytokines such as Tumor Necrosis Factor-alpha (TNF- α), Interleukin-1 beta (IL-1 β), Interleukin-6 (IL-6) and Interleukin-12 (IL-12), as well as anti-inflammatory cytokines such as Interleukin-10 (IL-10) and Transforming Growth Factor-beta (TGF- β) in the pathogenesis of tuberculosis. The review examines the effects of *M. tuberculosis* on cytokine responses that promote persistent infection, the importance of cytokine balance in forming and maintaining granulomas, and the role of cytokine dysregulation in severe disease manifestations. In addition, we discuss genetic polymorphisms in cytokine genes as determinants of susceptibility to contracting tuberculosis, as well as the effect of co-infection on the cytokine profiles and future therapeutic interventions focusing on cytokine pathways. It is essential to understand these complex cytokine-based mechanisms to devise new immunotherapeutic strategies and enhance the outcomes of the treatment of tuberculosis and other mycobacterial infections.

Keywords: Cytokines, *Mycobacterium tuberculosis*, Inflammation, Interleukins, TNF- α , granuloma, Immunopathology.

1. Introduction

1. 1. Global Impact of *Mycobacterium tuberculosis*

Mycobacterium tuberculosis (Mtb) has been among the major causes of morbidity and mortality worldwide, with an estimated 10 million cases and 1.5 million deaths occurring each year (Chakaya et al., 2021; *Global Tuberculosis Report*, 2024). Although there is now much more knowledge about tuberculosis (TB) pathogenesis, as well as the presence of effective antimicrobial therapy, the appearance of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has brought a pressing issue of the necessity to develop new therapeutic modalities (Dheda et al., 2017; Seung et al., 2015). The interplay between the Mtb and the host immune system is a very complicated phenomenon that can be considered a critical determinant of the disease outcome, and cytokines are one of the main mediators of this complex biological communication (Flynn & Chan, 2022; Philips & Ernst, 2012). Importantly, cytokine profiling among drug-resistant individuals has recently revealed exaggerated hyperinflammatory cytokine signatures associated with disease severity in recent cohorts, highlighting the need to consider cytokine biology in MDR/XDR-TB strategies (Zhang et al., 2023).

Mtb has been successful as a human pathogen due to its amazing power to survive and reproduce inside the host macrophages, which are the same cells that are meant to destroy the pathogens that invade them (Cambier et al., 2014). The intracellular lifestyle requires complex processes of immune evasion and control of host cell functions, primarily through the regulation of cytokine synthesis and signalling pathways (Kleinnijenhuis et al., 2011). Elucidating the role of cytokines in the immune response to Mtb infection is critical to the development of effective immunotherapies that can boost antimicrobial immunity while reducing tissue damage (Zumla et al., 2016).

1. 2. Cytokines: Definition and Classification

Cytokines are minuscule secreted proteins that serve as cell-to-cell messengers, mediating communication among immune cells and coordinating inflammatory and immune responses (Turner et al., 2014). These soluble mediators may be categorized according to their structure, cellular source or biological function. The general classification of cytokines in the case of bacterial infections is into pro-inflammatory cytokines, which induce antimicrobial effects and tissue inflammation, and anti-inflammatory cytokines, which control and resolve inflammatory reactions (Opal & DePalo, 2000).

Among the pro-inflammatory cytokines are tumor necrosis factor-alpha (TNF-alpha), interleukin-1 β , interleukin-6 (IL-6), interleukin-12 (IL-12), and interferon-gamma (IFN-gamma) (Parameswaran & Patial, 2010). These cytokines mobilise the immune cells, stimulate phagocytic functions, stimulate the attraction of leukocytes to infection sites, and develop adaptive immune responses. Pro-inflammatory reactions are neutralised by anti-inflammatory cytokines, such as interleukin-10 (IL-10) and transforming growth factor- β (TGF- β), which inhibit excessive tissue injury and help resolve inflammatory events (Couper et al., 2008).

The chemokine family is a distinct group of cytokines whose main action is to serve as chemoattractants to guide the movement of immune cells to the location of inflammation or infection (Charo & Ransohoff, 2006). Some of the important chemokines implicated in TB pathogenesis are CCL2 (MCP-1), CCL5 (RANTES), CXCL8 (IL-8), CXCL9, and CXCL10 (Zhuang et al., 2024). These molecules are important in the formation of granulomas, the recruitment of immunological cells, and the spatial arrangement of immune responses in infected tissues.

2. The Pathobiology of *Mycobacterium tuberculosis* Infection

The transmission of Mtb happens mainly by breathing in aerosol droplets containing the bacteria, which are lodged along the lung alveoli, where they interact with alveolar macrophages (Ernst, 2012). Host cells respond to the initial contact with Mtb through a cascade of events that helps in defining whether the infection will be suppressed, develop active disease or enter into

a state of latency (Flynn et al., 2011). This is a critical period of infection during which there will be excessive interaction between innate immune cells and a series of cytokines produced that influence further adaptive immune responses.

After being phagocytosed by alveolar macrophages, Mtb has several mechanisms of surviving in the hostile intracellular environment (Weiss & Schaible, 2015). The bacteria are able to prevent phagosome-lysosome fusion, control phagosomal pH, withstand oxidative and nitrosative stress, and regulate host cell death. These survival responses are closely connected with the capacity of the bacterium to disrupt the cytokine signalling, directly regulating the cytokine production or changing the response of the cell to the cytokine stimulation (Stanley & Cox, 2013).

The characteristic pathological process in the case of TB is the appearance of granulomas, which are organized formations of infected macrophages with the presence of other types of immune cells such as lymphocytes, neutrophils, fibroblasts and multinucleated giant cells (Ramakrishnan, 2012). The formation of granulomas is a host mechanism to localize the infection and avoid the spread of bacteria; yet, at the same time, it offers a niche under which Mtb can survive in a metabolically inactive form. The local cytokine milieu also has a significant impact on the development of granulomas and their ultimate outcome, and the knowledge of cytokine networks is critical to understanding the pathogenesis of TB (Gideon et al., 2015).

One of the most important aspects of granuloma biology, yet one that is frequently overlooked, is the polarization of macrophages in granulomas. Macrophages expressing a pro-inflammatory (M1) phenotype, stimulated by signaling pathways activated by IFN- γ and TNF- α , have enhanced antimicrobial activity, including the production of reactive oxygen and nitrogen species, the secretion of pro-inflammatory cytokines, and the upregulation of MHC class II molecules. However, alternatively activated (M2) macrophages, stimulated by IL-4, IL-13 and IL-10, have anti-inflammatory functions like tissue repair and fibrosis and reduced bactericidal activity. TNF- α and IFN- γ stimulate the M1 phenotype, which is associated with an inflammatory response, increasing bacterial clearance, while IL-10 and TGF- β stimulate the M2 repair phenotype. Mtb takes full advantage of this balance, inducing production of IL-10 to polarise macrophages to M2 in order to dampen antimicrobial responses and promote the persistence of infection in granulomas. Thus, understanding the regulation of macrophage polarization by cytokines is important for understanding the mechanism of granuloma formation, maintenance and ultimate disease outcome (Ahmad et al., 2022; Gideon et al., 2015).

3. Pro-Inflammatory Cytokines in TB Immunity

3. 1. Tumor Necrosis Factor-Alpha (TNF- α)

One of the most important cytokines in anti-mycobacterial immunity is TNF-alpha, and the relevance of this particular cytokine has been emphasized in a dramatic manner by the higher rates of TB reactivation in patients undergoing anti-TNF therapy for autoimmune disease (Keane et al., 2001). Activated T cells and macrophages are the major producers of this pleiotropic cytokine after they recognise Mtb pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) (Roach et al., 2002).

The multifunctional TNF- α plays multiple roles in TB immunity, including enhancing macrophage antimicrobial processes by stimulating phagosome maturation, triggering the production of reactive oxygen and nitrogen species, inducing autophagy, and regulating apoptosis (Divangahi et al., 2009). TNF- α is also a highly important consideration in the formation and maintenance of granulomas, which regulate the spatial arrangement of immune cells and eliminate the dissemination of bacteria (Marino et al., 2015). At the molecular level, TNF- α is signalled by two receptors, TNFR1 and TNFR2, which signal through different downstream pathways, including NF- κ B, MAPK and death receptor signalling cascades.

Mice lacking TNF- α have also been proved to have a strict need for this cytokine in Mtb infection control whereby knockout mice succumb to bacterial overload and spread the disease within a very short time (Flynn et al., 1995).

Nonetheless, the excessive production of TNF- α may lead to immunopathology, tissue necrosis, and cachexia; therefore, there is a need to regulate the levels of TNF- α that is produced during the infection with specific precision (Harris & Keane, 2010). This paradoxical effect of TNF- α as both a defensive and potentially harmful cytokine is an example of a narrow range of response needed to optimally protect against Mtb (Figure 1).

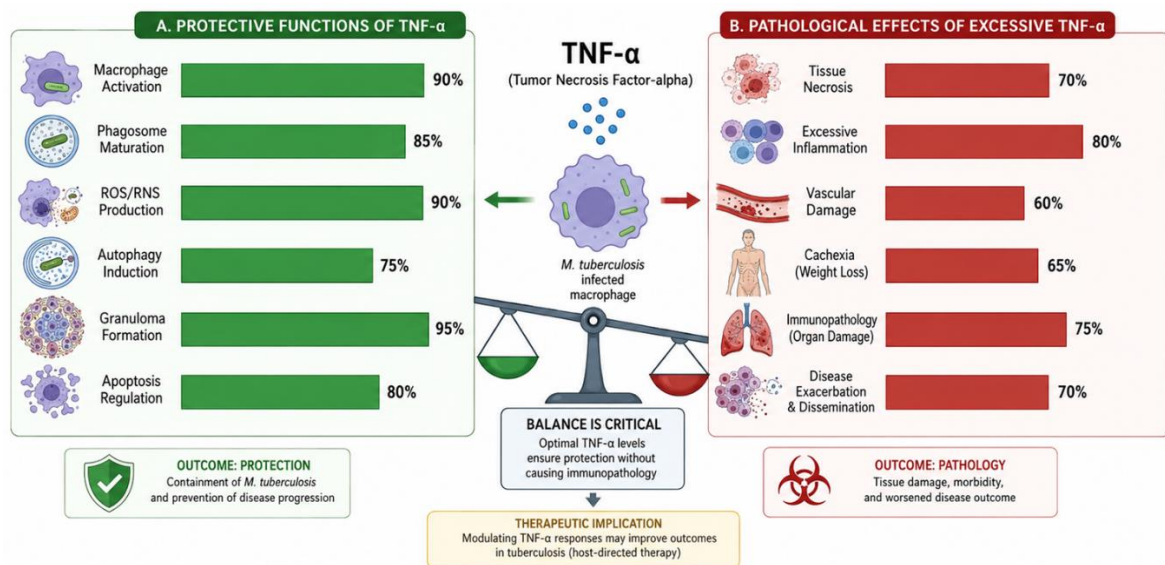


Figure 1. Dual role of TNF- α in TB immunity. TNF- α is important for the activation of macrophages, the development of granulomas, autophagy and controlling bacteria, but excessive levels of TNF- α can lead to inflammation, tissue necrosis and immunopathology. It is crucial to have a balanced response to TNF- α if you are to provide protection against *Mycobacterium tuberculosis*.

3. 2. Interleukin-1 B (IL-1 β)

Another important pivotal pro-inflammatory cytokine in anti-TB immunity refers to IL-1 β , which is mainly generated when the NLRP3 inflammasome is activated due to Mtb infection (Fremond et al., 2007). The inflammasome is a complex of multiple proteins that is able to detect pathogen-associated and danger-associated molecular patterns, which causes caspase-1 to be activated and pro-IL-1 β to be processed into its active, bioactive form. Mtb elements like the ESX-1 secretion apparatus and bacterial DNA have been found to be important inducers of inflammasome activation (K. D. Mayer-Barber et al., 2011).

Protective roles of IL-1 β in TB encompass improvement of antimicrobial effector functions, T helper 17 (Th17)-cell differentiation, eicosanoid synthesis and regulation of type I interferon responses, which are potentially pathological during Mtb infection and excessively activated (Mishra et al., 2013). The role of IL-1 β is also related to the fevers and the acute-phase response, which are the traditional symptoms of the TB disease. Researchers have found that the association of TB vulnerability with the polymorphism of IL1B exists in multiple populations, highlighting the clinical importance of the cytokine (K. Mayer-Barber et al., 2010).

Nevertheless, like TNF- α , IL-1 β activity also needs to be strictly regulated to avoid immunopathology. The natural antagonist, IL-1 receptor antagonist (IL-1Ra), is an endogenous antagonist that competes with IL-1 β in receptor binding but does not promote signalling. Dysregulation of the IL-1 β /IL-1Ra balance has been referred to as a cause of severe manifestations of TB and immune reconstitution inflammatory syndrome (IRIS) during the treatment (McGeachy et al., 2019).

3. 3. Interleukin-6 (IL-6)

IL-6 is a pleiotropic cytokine that has both pro-inflammatory and anti-inflammatory functions during *Mtb* infection (McGeachy et al., 2019). IL-6 is produced by the response of macrophages, dendritic cells and stromal cells against TLR signalling and other pro-inflammatory cytokines and has a significant role in the regulation of both innate and adaptive immunity. The cytokine binds to a hexameric IL-6/ IL-6 receptor (IL-6R) / gp130 receptor complex, leading to the activation of the JAK-STAT3 pathway, among other signalling pathways.

IL-6 plays a role in TB pathogenesis in the acute response, T cell activation and differentiation, production of antibodies, and regulation of local inflammatory response in granulomas (Saunders et al., 2000). It has always been shown in clinical studies that IL-6 levels are higher in patients with an active TB disease, which is associated with the severity of the disease and the quantity of bacteria. The levels of plasma IL-6 reduce after the effective completion of anti-TB treatment, suggesting that IL-6 could be used as a biomarker for treatment monitoring (Walzl et al., 2018). These findings have been reinforced by more recent multiplex cytokine panel studies, which identified IL-6 and other cytokines that responded to treatment as composite signatures that predicted poor treatment outcomes in pulmonary TB (Pandiarajan et al., 2024; Zhang et al., 2023).

The dualism of IL-6 is evident, bearing in mind its involvement in both protective immunity and immunopathology. Although IL-6 is required to maximise T cell response and maintenance of granuloma, a large production of IL-6 has been linked to tissue damage, cachexia, and systemic inflammation. It has also been shown that IL-6 can suppress Th1 responses while promoting Th17 differentiation, which complicates the role of IL-6 in anti-mycobacterial immunity.

3. 4. Interleukin-12 (IL-12) and the IL-12/IFN- γ Axis

IL-12 is an important link between innate and adaptive immunity in TB, as it is mainly produced by antigen-presenting cells such as macrophages and dendritic cells after the recognition of *Mtb* (Trinchieri, 2003). It is a heterodimeric cytokine composed of the p35 and p40 subunits and is a strong stimulator of IFN- γ production by the NK cells and T cells. The IL-12/IFN- γ axis can be deemed the backbone of protective immunity against *Mtb*, wherein genetic mutations in the IL-12/IFN- γ pathway lead to a highly susceptible nature to mycobacterium infections (Ottenhoff et al., 1998).

The relevance of IL-12 in TB immunity has been supported by the fact that IL-12-deficient mice have defective Th1 responses, decreased production of IFN- γ , poor granuloma formation, and a failure to contain bacterial replication (Cooper et al., 1993). In human beings, gene mutations in IL-12 or its receptor (IL12RB1) cause Mendelian susceptibility to mycobacterial disease (MSMD), which are the severe infections caused by environmental mycobacteria and BCG vaccine strains.

The biological activity of IL-12 acting on the IL-12 receptor (IL-12R), which is composed of two chains, namely IL-12R β 1 and IL-12R β 2, causes the stimulation of STAT4 and the resultant activation of the IFN- γ gene and other Th1-like genes. In addition to its effect on IFN- γ , IL-12 stimulates NK cell cytotoxicity, stimulates T cell growth and survival, and balances the different T helper cell subsets. The resulting IFN- γ induced by IL-12 further induces a feedback effect on the macrophage antimicrobial capacities and forms a positive feedback mechanism critical in controlling bacteria (Figure 2).

3. 5. Interferon-Gamma (IFN- γ)

The prototypical Th1 cytokine and arguably the most critical effector of Th1 immunity against *Mtb* is IFN- γ (Schroder et al., 2004). IFN- γ is the main macrophage-sensitising factor, produced mainly by CD4⁺ and CD8⁺ T cells and NK cells. It causes a programme of antimicrobial reactions that involve heightened phagolysosome maturation, augmented production of reactive oxygen and nitrogen intermediates, amplified autophagy, and augmented expression of the MHC class II (Ehrt et al., 2001).

The contribution of IFN- γ to TB immunity has been clearly demonstrated in experiments on IFN- γ -KO mice, which immediately become infected with *Mtb*, with active bacterial multiplication and spreading of the disease (Flynn et al., 1993).

On the same note, humans lacking IFN- γ or its receptor (IFNGR1 or IFNGR2) genetically develop serious infections of mycobacteria, which confirms again the non-redundant role of this cytokine. Interestingly, although IFN- γ is vital in the early management of Mtb infection, high concentrations that persistently rise may contribute to tissue damage and disease progression, highlighting the value of proper regulation.

IFN- γ expresses the IFN- γ receptor (IFNGR), a heterodimeric receptor complex of IFNGR1 and IFNGR2 chains, which triggers the JAK-STAT1 pathway with hundreds of interferon-stimulated genes (ISGs) activated. Some of these ISGs include genes that encode antimicrobial effector molecules, antigen presentation machinery, and cellular metabolic regulators. The magnitude and duration of IFN- γ signalling are maintained at various levels: receptor expression, negative regulators including SOCS proteins and epigenetic changes.

Table 1. Major Pro-Inflammatory Cytokines in M. tuberculosis Infection.

Cytokine	Primary Sources	Key Receptors	Main Functions in TB	Signalling Pathways	Clinical Significance
TNF-α	Macrophages, T cells	TNFR1, TNFR2	Granuloma formation, macrophage activation, apoptosis regulation	NF- κ B, MAPK, death receptor	Essential for control; anti-TNF therapy increases TB risk
IL-1β	Macrophages, monocytes	IL-1R1	Inflammasome activation, Th17 differentiation, eicosanoid production	MyD88, NF- κ B	Polymorphisms associated with susceptibility
IL-6	Macrophages, DCs, stromal cells	IL-6R/gp130	Acute phase response, T cell activation, antibody production	JAK-STAT3, MAPK	Elevated in active disease; biomarker potential
IL-12	Macrophages, DCs	IL-12R β 1/ β 2	IFN- γ induction, Th1 differentiation, NK cell activation	JAK-TYK2-STAT4	Deficiency causes MSMD
IFN-γ	T cells, NK cells	IFNGR1/IFNGR2	Macrophage activation, autophagy, ROS/RNS production	JAK-STAT1	Essential for protection; genetic deficiency lethal

4. Anti-inflammatory and Regulatory Cytokines

4. 1. Interleukin-10 (IL-10)

The prototype of anti-inflammatory cytokine is IL-10, which is an important but complex element of TB pathogenesis (Redford et al., 2011). Although IL-10 is crucial in inhibiting excessive inflammation and tissue damage, it may also suppress antimicrobial immunity as well as promote bacterial persistence. It is produced by other cell types such as macrophages, dendritic cells, regulatory T cells (Tregs) and even infected cells that react to Mtb, indicating that there is more than one cell layer of immune control (Sendide et al., 2005).

The immunosuppressive effects of IL-10 occur via the IL-10 receptor (IL-10R), which stimulates the activation of the STAT3 signalling pathway, leading to gene expression that inhibits inflammatory responses. IL-10 suppresses the expression of pro-inflammatory cytokines such as TNF- α , IL-1 β , IL-6, and IL-12, as well as the expression of MHC class II and

costimulatory molecules on antigen-presenting cells, T cell activation and proliferation (Moore et al., 2001). Considering the case of Mtb infection, IL-10 can suppress phagosome-lysosome fusion through precise molecular mechanisms. IL-10-mediated STAT3 activation inhibits calcium signalling required for phagosomal maturation and prevents the proper recruitment and aggregation of key endosomal maturation markers, including EEA1 (Early Endosome Antigen 1) and Rab7, on the phagosomal membrane. This blockade of EEA1 and Rab7 recruitment prevents late endosome-lysosome fusion, thereby halting the acidification and enzymatic degradation necessary to kill intracellular Mtb. In addition, IL-10 decreases the production of reactive nitrogen species and inhibits autophagy, thus helping bacteria to survive.

In vivo experiments with IL-10-deficient mice have produced apparently contradictory findings, with some studies reporting improved bacterial control and others a higher incidence of immunopathology and death (Beamer et al., 2008). Such divergent results indicate the context dependence of IL-10 activity and indicate that the timing, amount, and cellular source of IL-10 production probably play a pivotal role in the outcome of a disease. Clinical studies have demonstrated increased levels of IL-10 in patients with active TB, and a high level of the IL-10/IFN- γ ratio has been attributed to poor prognosis. Mtb has developed mechanisms to bypass IL-10-mediated immunosuppression, whereby some secreted factors and bacterial components can induce IL-10 (Hmama et al., 2015). Such IL-10 induction by this pathogen can be an immune evasion mechanism of bacteria, thus permitting Mtb to become a chronic infection by suppressing protective immunity (Figure 2).

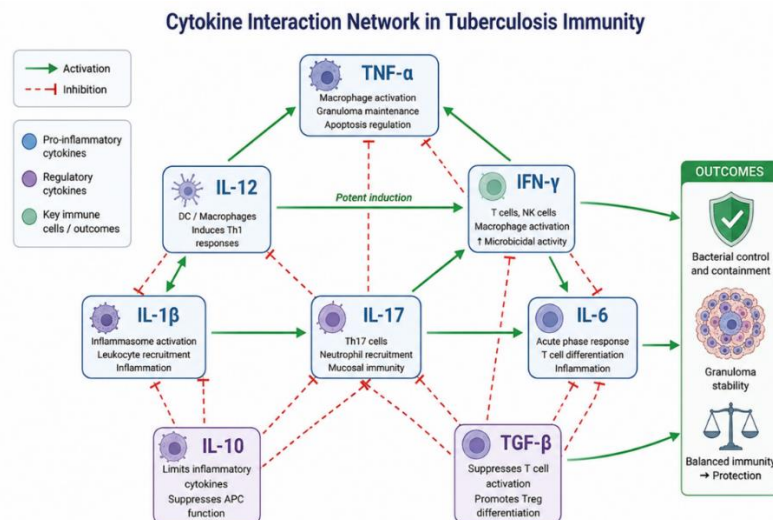


Figure 2. Immunity against tuberculosis, mediated by cytokines. Green arrows mean activation, red dashed lines mean inhibition. The IL-12/IFN- γ axis is linked to the protective immunity, and IL-10 and TGF- β are linked to excess inflammation.

4. 2. Transforming Growth Factor-B (TGF- β)

Another immunoregulatory cytokine is TGF- β , which has divergent functions in TB pathogenesis (Deng et al., 2024). TGF- β is a cytokine that has three mammalian isoforms (TGF- β 1, TGF- β 2, and TGF- β 3) and is secreted by a wide range of cell types such as macrophages, T cells, and stromal cells. TGF- β binds to serine/threonine kinase receptors (TGF β RI and TGF β RII), which stimulate SMAD-dependent and SMAD-independent pathways.

TGF- β , in the case of Mtb infection, has immunosuppressive properties such as T cell growth and activation, macrophage activation, production of pro-inflammatory cytokines, and regulatory T cell differentiation (Toossi et al., 1995). TGF- β can also affect tissue remodelling and fibrosis, which are also pertinent to the formation of the granuloma and cavitation in TB. High levels of TGF- β have been observed in tuberculous pleural effusions and in lung lesions, indicating that there is active immunoregulation in areas of infection.

Nevertheless, TGF- β is not always harmful to TB immunity. It is a cytokine that helps maintain immune homeostasis and prevents overproduction of inflammation that may result in tissue damage. It also plays a role in applying roles in the healing of inflammation following successful treatment. The proportion between TGF- β and pro-inflammatory cytokines is probably the requirement to regulate immune responses properly or suppress them pathologically (Kuroda et al., 2018).

5. Chemokines and Immune Cell Recruitment

5. 1. C-C Chemokine Ligands (CCL)

The spatial organisation of immune responses is achieved through the coordination of the movement of certain populations of cells to the location of infection under the direction of the chemokines (Griffith et al., 2014). There are various C-C chemokine ligands that are critical in the recruitment of immune cells and formation of granuloma in TB. One of the most highly expressed chemokines in response to Mtb infection is CCL2 (MCP-1) that attracts monocytes and macrophages to infected tissue (Dorhoi & Kaufmann, 2016). CCL2 has been observed to be elevated in serum, bronchoalveolar lavage fluid, and tissue samples of individuals with TB.

Other significant C-C chemokines in TB immunity include CCL3 (MIP-1 α), CCL4 (MIP-1 β), and CCL5 (RANTES), which attract T cells, NK cells, and monocytes into granulomas (Algood et al., 2004). Activated macrophages and dendritic cells produce these chemokines in the presence of Mtb and pro-inflammatory cytokines. Research with chemokine knockout mice has shown cellular recruitment of the immune cells, lack of granuloma formation, and greater vulnerability of the organism to Mtb infection.

Chemokine gradients, which are formed through the coordinated expression of several chemokines, help direct the various cell types within granulomas to distinct areas, and this is part of the organised structure of such lesions. Nevertheless, chemokine secretion may cause immunopathology with the excessive recruitment of inflammatory cells, leading to tissue destruction in case of overproduction (Gopal et al., 2013). Striking the right balance between the right cell recruitment and pathological inflammation is thus essential in the realisation of the best TB results (Figure 3).

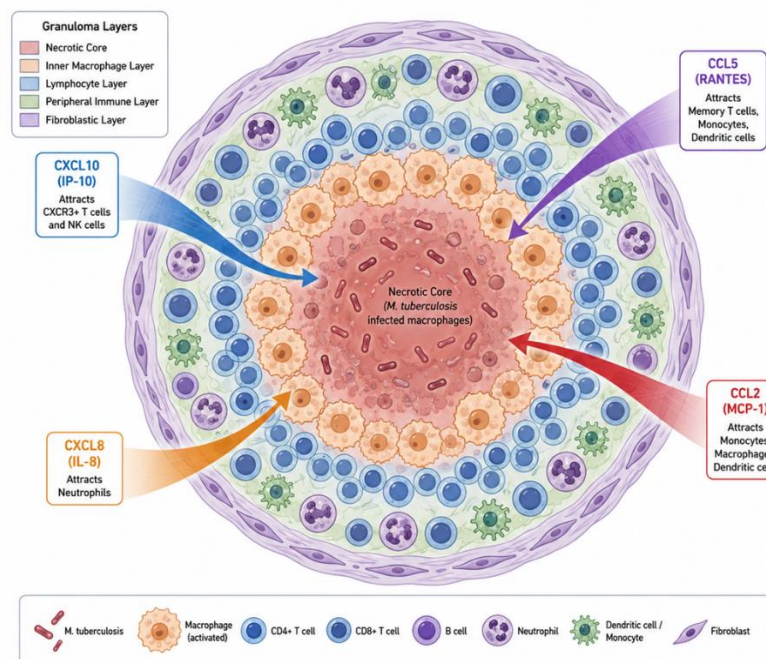


Figure 3. Chemokine-mediated cell recruitment in the tuberculosis granuloma. Chemokine gradients coordinate immune cell trafficking and granuloma organisation during *Mycobacterium tuberculosis* infection.

5. 2. C-X-C Chemokine Ligands (CXCL)

Another family of chemokines that plays an important role in the pathogenesis of TB is the C-X-C chemokine ligands. CXCL8 (IL-8) is a strong neutrophil chemoattractant produced in large amounts during Mtb infection (Nouailles et al., 2014). Although neutrophils are involved in the initial antimicrobial reactions, neutrophil hyper-recruitment and hyper-activation have been linked to tissue destruction and adverse disease outcomes in TB.

The induction of CXCL9, CXCL10, and CXCL11 (also known as interferon-inducible CXC chemokines) by IFN-gamma plays significant roles in targeting activated T cells and NK cells to Mtb infection foci (Antonelli et al., 2010). These chemokines communicate via the CXCR3 receptor and help in the formation and elimination of granulomas. CXCL9 and CXCL10 have been detected in active TB patients and have been found promising as diagnostic and prognostic biomarkers.

Table 2. Key Chemokines in Tuberculosis Pathogenesis.

Chemokine	Alternative Names	Primary Targets	Receptor	Functions in TB	Clinical Observations
CCL2	MCP-1	Monocytes, macrophages	CCR2	Monocyte recruitment, granuloma formation	Elevated in active TB
CCL5	RANTES	T cells, eosinophils	CCR1, CCR3, CCR5	T cell recruitment, granuloma maintenance	Elevated in TB pleurisy
CXCL8	IL-8	Neutrophils	CXCR1, CXCR2	Neutrophil recruitment	High levels in severe disease
CXCL10	IP-10	Activated T cells, NK cells	CXCR3	Th1 cell recruitment	Diagnostic potential

6. Cytokine Networks and Cross-regulation

6. 1. The Th1/Th2 Balance

T helper 1 (Th1)-T helper 2 (Th2) balance is a well-known paradigm in immunology that is of great importance to the pathogenesis of TB (Lyadova & Panteleev, 2015). Th1 responses, which involve the production of IFN-gamma, TNF-alpha and IL-2, favour cell-mediated immunity and the activation of macrophages necessary in the process of controlling intracellular pathogens such as Mtb. Conversely, Th2 responses, characterized by the production of IL-4, IL-5 and IL-13, are usually linked to humoral immunity and allergy.

In Mtb infection, Th1 is the essential response that is important in offering protective immunity (Brighenti & Andersson, 2012). Antigen-presenting cells produce IL-12, which induces Th1 differentiation by activating the STAT4 pathway, and IL-4 stimulates Th2 differentiation by activating the STAT6 pathway. There is antagonism between these pathways, where IFN- γ suppresses Th2 responses, and IL-4 suppresses Th1 differentiation. A change towards a Th2 cytokine profile has been linked to progressive TB disease, suggesting that this imbalance has a role in failed immunity.

6. 2. The Th17/Treg Axis

The Th17/Treg axis is the other important axis in TB immunity (Cruz et al., 2006). Th17 cells play an important part in mucosal immunity, neutrophil recruitment, and antimicrobial peptide production because of their production of IL-17A, IL-17F, and IL-22. TGF- β together with pro-inflammatory cytokines like IL-6, IL-1 β , and IL-23 induces these cells.

Th17 responses have been implicated in protection and pathology based on the situation in TB (Khader et al., 2007). Recruitment of neutrophils through IL-17 can facilitate a rapid response to bacteria, whereas a large response by Th17 cells can lead to tissue destruction and cavitation. Remarkably, the development of Th17 and Treg cells is similar, and TGF- β is necessary for both lineages; however, the final cell fate is predetermined by the presence of pro-inflammatory cytokines.

The Th17/Treg balance is dynamically maintained in the case of TB infection, and changes in this balance may affect the course of disease and treatment outcomes (Torrado & Cooper, 2010). Some studies have demonstrated that active TB is associated with an increased Th17 response, which resolves with proper treatment, whereas others have demonstrated high Treg frequencies knocking out protective immunity. The Th17/Treg cell ratio has been suggested as a possible biomarker of disease condition and response to treatment.

6. 3. Type I Interferon Responses

Type I interferons (IFN- α/β) are a group of cytokines that are traditionally linked to antiviral immune response but have recently been found to be key regulators of TB disease progression (Berry et al., 2010). In contrast to the protective effects of IFN- γ (type II interferon), type I interferons may be harmful during Mtb infection in some circumstances. Overactivity of type I interferon signalling has been demonstrated to inhibit protective Th1 responses, maladapt Th1 macrophage antimicrobial responses, and induce vulnerability to TB in mouse models.

The pathways of these damaging impacts of type I interferons in TB include the inhibition of IL-12 and IL-10 production, stimulation of cell death leading to the release of bacteria from infected cells, and direct inhibition of IFN- γ signalling (Mundra et al., 2023). Interestingly, the exploitation of type I interferon responses by Mtb may be active, and there are some bacterial components that tend to induce this pathway (Figure 2).

7. Cytokine Dysregulation and Disease Manifestations

7. 1. Cytokine Storms and Severe TB

The cytokine storm, which is characterised by the uncontrolled and excessive production of the pro-inflammatory cytokines, is a severe complication of TB that may result in acute respiratory distress syndrome (ARDS), septic shock, and multi-organ failure (Channappanavar & Perlman, 2017). The condition is hyperinflammatory and promoted by positive feedback loops of TNF- α , IL-1 β , and IL-6 among other pro-inflammatory cytokines, leading to systemic inflammation and tissue destruction (Figure 4).

Dysregulated cytokine reaction is especially linked with certain types of TB, such as miliary TB and TB meningitis. The pro-inflammatory cytokine versus anti-inflammatory cytokine balance is, in such instances, grossly disturbed, resulting in immunopathology that is a leading cause of morbidity and mortality (Sia et al., 2015). It is fundamental to understand the causes and pathophysiology of cytokine storms in TB when designing measures to curb or attenuate these life-threatening complications.

7. 2. Immune Reconstitution Inflammatory Syndrome (IRIS)

TB-related IRIS develops in a group of patients after they take antiretroviral therapy during HIV-TB co-infection or when they take anti-TB medication in severely immunocompromised patients (Meintjes et al., 2008). It is a paradoxical exacerbation of clinical symptoms with the increase in microbiological improvement, mediated by the rapid reconstitution of pathogen-specific immune responses, in particular, the exaggerated production of pro-inflammatory cytokines.

It has been observed that the levels of TNF- α , IFN- γ , IL-6 and IL-18 are significantly higher in IRIS patients with inadequate regulation of cytokines such as IL-10. The IRIS cytokine profile indicates the presence of an unbalanced immune reconstitution

process in which the pro-inflammatory processes surpass regulatory processes. Targeted therapeutic interventions (i.e., TNF- α and IL-6) have been shown to be promising therapeutic options in treating severe cases of IRIS.

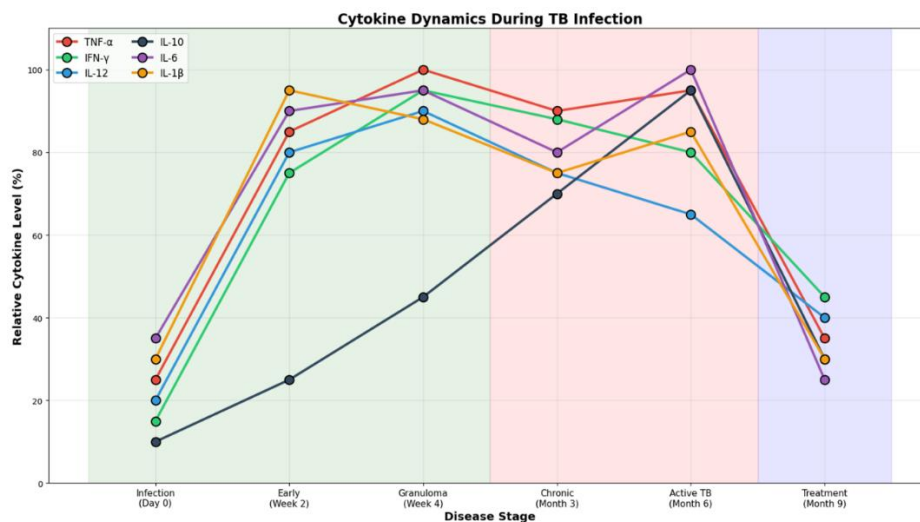


Figure 4. Cytokine temporality during the TB infection and treatment. This line graph will represent the relative concentrations of key cytokines (TNF- α , IFN- γ , IL-12, IL-10, IL-6, IL-1 β) in six stages of disease: initial infection, early response, granuloma formation, chronic disease, active disease, and treatment disease. The graph is broken down into three phases that are represented by the shaded areas, namely, protective phase (green), pathological phase (red), and recovery phase (blue). The peak levels of pro-inflammatory cytokines are observed in the initial response phase and are sustained in the active disease phase, whereas the IL-10 levels rise gradually in the chronic and active disease phases. The outcome of the treatment is the recovery of normal cytokine levels. It is a significant pattern that can be used to consider the timing of immunotherapeutic interventions.

7. 3. Genetic Polymorphisms and Cytokine Responses

The individual cytokine gene variations have been major contributors to individual vulnerability to TB and disease outcomes (Schurz et al., 2024). Genomic evidence contributing to changes in TB risk includes single nucleotide polymorphisms (SNPs) in TNF- α , IL-10, IL-12, and IFN- γ , always with their respective receptors. These polymorphisms may change cytokine production levels, receptor affinity and the efficiency of the signalling process.

As an example, the TNF- α -308G/A polymorphism has been implicated in a higher risk of TB among certain populations, perhaps by placing TNF- α under the influence of changed production (Correa et al., 2005). In a similar manner, polymorphisms of IL-10 promoters leading to increased IL-10 production have been linked to an increased risk of TB, which is consistent with the immunosuppressive effects of this cytokine. Knowledge of the genetic etiology of cytokine dysregulation may facilitate individualized medicine in the treatment of TB.

8. Therapeutic Targeting of Cytokine Pathways

8. 1. Current Immunotherapeutic Approaches

The immunobiological importance of cytokines in TB immunity has led to interest in immunotherapeutic strategies to regulate cytokine responses to promote better control of this organism but with limited immunopathology (Guler et al., 2021). Some of the strategies that are being studied include cytokine supplementation, cytokine neutralisation, and alteration of cytokine signalling pathways.

The use of IFN- γ therapy as an adjunctive therapy to treat drug-resistant TB and patients with compromised immunity has also been investigated, with some clinical improvements being reported. On the other hand, countering any undesirable IL-10 or TGF- β to boost antimicrobial immunity may be another approach, but one should pay attention to preventing immunopathology. The intake of particular cytokine suppressors like anti-TNF- α agents is extremely precarious because of the danger of the reactivation of TB (Figure 5).

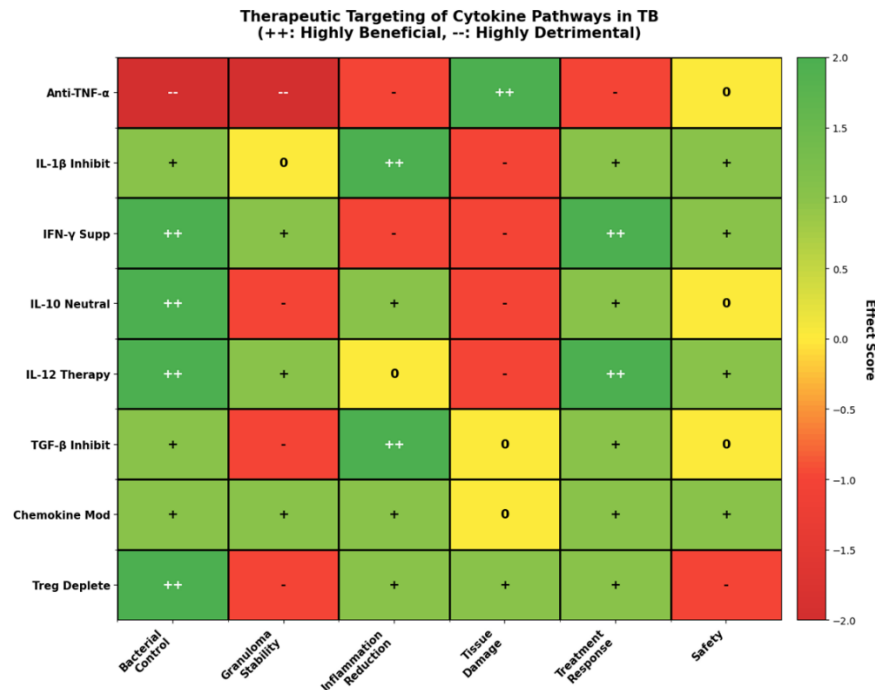


Figure 5. Cytokine pathway therapeutic targeting of TB. Heatmap to illustrate the predicted effectiveness of the various immunotherapeutic strategies in different outcomes. Anti-TNF- α blockade, IL-1 β neutralisation, IFN- γ treatment, IL-10 neutralisation, IL-12 therapy, TGF- β inhibition, chemokine, and Treg depletion can be used as strategies. Bacterial control, granuloma stability, reduction in inflammation, tissue damage, treatment response and the safety profile are the outcomes that are assessed. There is the color coding of red (highly detrimental), yellow (neutral) and also green (highly beneficial). The heatmap demonstrates that anti-TNF- α blockade is extremely harmful to the level of bacterial control and granuloma stability, whereas such strategies as IFN- γ supplementation and IL-12 therapy have a very positive impact. This discussion offers a paradigm for the rational choice of cytokine-based therapeutic interventions in TB.

8. 2. Host-Directed Therapies

Instead of attacking the pathogen directly, host-directed therapies (HDTs) attempt to mediate host immune responses (Wallis et al., 2016). The benefits associated with these methods are that the risk of drug resistance is minimised and there is a possible effectiveness of the treatments against drug-resistant strains. Some of the HDTs currently being studied by researchers include cytokine pathway modulation, such as COX-2 inhibitors that regulate eicosanoid-mediated inflammation, JAK-STAT inhibitors, and inflammasome activation.

Recycling already available drugs which have immunomodulatory effects is a good approach toward expedited application to clinical practice. Metformin, statins and even vitamin D have been shown to have the potential to positively regulate cytokine responses in TB. It is important to understand the cytokine-modulating properties of these agents to use them in the best way possible (Singhal et al., 2014).

8. 3. Future Directions and Challenges

Combination modalities that concurrently inhibit multiple cytokine mediators, thereby providing an ideal balance of immunity, are likely to be the subject of immunotherapeutic techniques in the future. Individualized medicine (based on individual cytokine levels and genetic backgrounds) could be used to provide TB patients with immunotherapy.

The main difficulties are determining biomarkers to predict which patients will respond to certain immunotherapies, finding the most appropriate timing of interventions, and coming up with a way through which the responses to therapies could be monitored. Systems biology methods and further development of immunological profiling will be crucial in the development of cytokine-directed therapy in TB.

9. Critical Analysis and Current Limitations

The data presented above reveal a consistent pattern of cytokines and their networks in TB, but many of them come from murine gene knockout models and in vitro macrophage systems, which do not adequately replicate the structure of the human granuloma or the genetic variability of *Mtb* strains in clinical settings. This is exemplified by IL-10-deficient mice, which have been shown to have improved bacterial control in one study, no effect in others, and even worse outcomes in some. It is clear that the protective-versus-pathological balance of a particular cytokine depends on its timing, its cell of origin, and its local bacterial burden and is not a fixed, uniform property of the molecule itself. However, results obtained in one model system need to be carefully extrapolated to human disease and require independent confirmation.

Another caveat relates to the translational bottleneck between mechanistic insights and clinical utility. The majority of the host-directed, cytokine-targeting strategies mentioned here are either preclinical or based on only small case series, with a few performed in well-powered randomized trials. This gap is becoming increasingly important as MDR/XDR-TB expands, because recent cytokine profiling studies have revealed hyperinflammatory signatures in drug-resistant disease, but have not yet confirmed whether these signatures are causal drivers that are safe to target or markers of disease severity ([Sampath et al., 2023](#)). Lastly, there is no consensus or external validation of a cytokine biomarker panel. Although there are promising candidates, which remain a major hurdle in the ability to translate cytokine biology into clinical use ([Pandiarajan et al., 2024](#); [Zhang et al., 2023](#)).

10. Future Directions

High-resolution characterization of cytokine networks has become more possible than ever, thanks to recent developments in single-cell RNA sequencing, spatial transcriptomics, and systems immunology. These technologies could enable the design of new treatments that act on the host and are tailored to the individual patients' needs, while also enhancing the ability to predict response to treatment.

11. Conclusion

Cytokines are essential intermediates of immunity against *Mycobacterium tuberculosis* and coordinate highly intricate networks of cell interactions that dictate the outcomes of the disease. The balance between pro-inflammatory and anti-inflammatory cytokines, which stimulate and inhibit immunopathology, respectively, is key to successful control of TB. The concept of a complex network of interactions of individual cytokines and the effects of their dysregulation has contributed to a better understanding of TB pathogenesis.

Recent breakthroughs have shown that *Mtb* actively controls host cytokine responses to create persistent infection, which shows the highly developed measures of immune evasion that this pathogen uses. There are context-dependent activities of

cytokines that are dependent on the stage of infection, bacterial load, and host genotype as well as co-infection, adding an additional layer of complication that needs to be taken into consideration in therapeutic schemes.

The development of immunotherapeutic interventions that focus on cytokine pathways is also a positive outcome in enhancing TB treatment outcomes, especially in cases of drug resistance. Nevertheless, one should take into account the proper balance of immune reactions, as both cytokine overstimulation and insufficient activation may lead to negative consequences.

Future research efforts are advised to aim at: (1) clarifying the exact timing and spatial patterns of cytokine generation in the presence of TB infection; (2) determining biomarkers influencing individual reactions to cytokine therapy; (3) creating an individualised methodology of immunotherapy and antimicrobial therapy based on host genetic and immunological indicators; and (4) combining cytokine regulation with standard antimicrobial therapy as a means of improving therapy efficacy and shortening of therapy.

With the current development of information about cytokine-mediated immunity in TB, the translation of this information into clinical practice is very promising as a way of dealing with the global TB epidemic, especially in the face of drug-resistant disease and HIV-TB co-infection. The overall knowledge acquired about cytokine networks will play a significant role in the development of the next-generation immunotherapies and vaccines capable of shifting the balance in favor of the host and ultimately contributing to the global eradication of tuberculosis.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

References

- Ahmad, F., Rani, A., Alam, A., Zarin, S., Pandey, S., Singh, H., Hasnain, S. E., & Ehtesham, N. Z. (2022). Macrophage: A Cell With Many Faces and Functions in Tuberculosis. *Frontiers in Immunology*, 13, 747799. <https://doi.org/10.3389/fimmu.2022.747799>
- Algood, H. M. S., Lin, P. L., Yankura, D., Jones, A., Chan, J., & Flynn, J. L. (2004). TNF Influences Chemokine Expression of Macrophages In Vitro and That of CD11b+ Cells In Vivo during Mycobacterium tuberculosis Infection. *The Journal of Immunology*, 172(11), 6846–6857. <https://doi.org/10.4049/jimmunol.172.11.6846>
- Antonelli, L. R. V., Gigliotti Rothfuchs, A., Gonçalves, R., Roffê, E., Cheever, A. W., Bafica, A., Salazar, A. M., Feng, C. G., & Sher, A. (2010). Intranasal Poly-IC treatment exacerbates tuberculosis in mice through the pulmonary recruitment of a pathogen-permissive monocyte/macrophage population. *Journal of Clinical Investigation*, 120(5), 1674–1682. <https://doi.org/10.1172/JCI40817>
- Beamer, G. L., Flaherty, D. K., Assogba, B. D., Stromberg, P., Gonzalez-Juarrero, M., De Waal Malefyt, R., Vesosky, B., & Turner, J. (2008). Interleukin-10 Promotes Mycobacterium tuberculosis Disease Progression in CBA/J Mice. *The Journal of Immunology*, 181(8), 5545–5550. <https://doi.org/10.4049/jimmunol.181.8.5545>
- Berry, M. P. R., Graham, C. M., McNab, F. W., Xu, Z., Bloch, S. A. A., Oni, T., Wilkinson, K. A., Banchereau, R., Skinner, J., Wilkinson, R. J., Quinn, C., Blankenship, D., Dhawan, R., Cush, J. J., Mejias, A., Ramilo, O., Kon, O. M., Pascual, V., Banchereau, J., ... O'Garra, A. (2010). An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. *Nature*, 466(7309), 973–977. <https://doi.org/10.1038/nature09247>
- Brighenti, S., & Andersson, J. (2012). Local Immune Responses in Human Tuberculosis: Learning From the Site of Infection. *The Journal of Infectious Diseases*, 205(suppl_2), S316–S324. <https://doi.org/10.1093/infdis/jis043>
- Cambier, C. J., Falkow, S., & Ramakrishnan, L. (2014). Host evasion and exploitation schemes of Mycobacterium tuberculosis. *Cell*, 159(7), 1497–1509. <https://doi.org/10.1016/j.cell.2014.11.024>
- Chakaya, J., Khan, M., Ntouni, F., Aklillu, E., Fatima, R., Mwaba, P., Kapata, N., Mfinanga, S., Hasnain, S. E., & Katoto, P. D. (2021). Global Tuberculosis Report 2020–Reflections on the Global TB burden, treatment and prevention efforts. *International Journal of Infectious Diseases*, 113, S7–S12. <https://doi.org/https://doi.org/10.1016/j.ijid.2021.02.107>
- Channappanavar, R., & Perlman, S. (2017). Pathogenic human coronavirus infections: Causes and consequences of cytokine storm and immunopathology. *Seminars in Immunopathology*, 39(5), 529–539. <https://doi.org/10.1007/s00281-017-0629-x>
- Charo, I. F., & Ransohoff, R. M. (2006). The Many Roles of Chemokines and Chemokine Receptors in Inflammation. *New England Journal of Medicine*, 354(6), 610–621. <https://doi.org/10.1056/NEJMra052723>
- Cooper, A. M., Dalton, D. K., Stewart, T. A., Griffin, J. P., Russell, D. G., & Orme, I. M. (1993). Disseminated tuberculosis in interferon gamma gene-disrupted mice. *The Journal of Experimental Medicine*, 178(6), 2243–2247. <https://doi.org/10.1084/jem.178.6.2243>
- Correa, P. A., Gomez, L. M., Cadena, J., & Anaya, J.-M. (2005). Autoimmunity and tuberculosis. Opposite association with TNF polymorphism. *The Journal of Rheumatology*, 32(2), 219.
- Couper, K. N., Blount, D. G., & Riley, E. M. (2008). IL-10: The master regulator of immunity to infection. *The Journal of Immunology*, 180(9), 5771–5777. <https://doi.org/https://doi.org/10.4049/jimmunol.180.9.5771>
- Cruz, A., Khader, S. A., Torrado, E., Fraga, A., Pearl, J. E., Pedrosa, J., Cooper, A. M., & Castro, A. G. (2006). Cutting Edge: IFN- γ Regulates the Induction and Expansion of IL-17-Producing CD4 T Cells during Mycobacterial Infection. *The Journal of Immunology*, 177(3), 1416–1420. <https://doi.org/10.4049/jimmunol.177.3.1416>
- Deng, Z., Fan, T., Xiao, C., Tian, H., Zheng, Y., Li, C., & He, J. (2024). TGF- β signaling in health, disease and therapeutics. *Signal Transduction and Targeted Therapy*, 9(1), 61. <https://doi.org/10.1038/s41392-024-01764-w>

- Dheda, K., Gumbo, T., Maartens, G., Dooley, K. E., McNerney, R., Murray, M., Furin, J., Nardell, E. A., London, L., & Lessem, E. (2017). The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. *The Lancet Respiratory Medicine*, 5(4), 291–360. [https://doi.org/https://doi.org/10.1016/S2213-2600\(17\)30079-6](https://doi.org/https://doi.org/10.1016/S2213-2600(17)30079-6)
- Divangahi, M., Chen, M., Gan, H., Desjardins, D., Hickman, T. T., Lee, D. M., Fortune, S., Behar, S. M., & Remold, H. G. (2009). *Mycobacterium tuberculosis* evades macrophage defenses by inhibiting plasma membrane repair. *Nature Immunology*, 10(8), 899–906. <https://doi.org/10.1038/ni.1758>
- Dorhoi, A., & Kaufmann, S. H. E. (2016). Pathology and immune reactivity: Understanding multidimensionality in pulmonary tuberculosis. *Seminars in Immunopathology*, 38(2), 153–166. <https://doi.org/10.1007/s00281-015-0531-3>
- Ehrt, S., Schnappinger, D., Bekiranov, S., Drenkow, J., Shi, S., Gingeras, T. R., Gaasterland, T., Schoolnik, G., & Nathan, C. (2001). Reprogramming of the Macrophage Transcriptome in Response to Interferon- γ and *Mycobacterium tuberculosis*. *The Journal of Experimental Medicine*, 194(8), 1123–1140. <https://doi.org/10.1084/jem.194.8.1123>
- Ernst, J. D. (2012). The immunological life cycle of tuberculosis. *Nature Reviews Immunology*, 12(8), 581–591. <https://doi.org/https://doi.org/10.1038/nri3259>
- Flynn, J. L., & Chan, J. (2022). Immune cell interactions in tuberculosis. *Cell*, 185(25), 4682–4702. <https://doi.org/10.1016/j.cell.2022.10.025>
- Flynn, J. L., Chan, J., & Lin, P. L. (2011). Macrophages and control of granulomatous inflammation in tuberculosis. *Mucosal Immunology*, 4(3), 271–278. <https://doi.org/https://doi.org/10.1038/mi.2011.14>
- Flynn, J. L., Chan, J., Triebold, K. J., Dalton, D. K., Stewart, T. A., & Bloom, B. R. (1993). An essential role for interferon gamma in resistance to *Mycobacterium tuberculosis* infection. *The Journal of Experimental Medicine*, 178(6), 2249–2254. <https://doi.org/10.1084/jem.178.6.2249>
- Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., Schreiber, R., Mak, T. W., & Bloom, B. R. (1995). Tumor necrosis factor- α is required in the protective immune response against *mycobacterium tuberculosis* in mice. *Immunity*, 2(6), 561–572. [https://doi.org/10.1016/1074-7613\(95\)90001-2](https://doi.org/10.1016/1074-7613(95)90001-2)
- Fremond, C. M., Togbe, D., Doz, E., Rose, S., Vasseur, V., Maillat, I., Jacobs, M., Ryffel, B., & Quesniaux, V. F. J. (2007). IL-1 Receptor-Mediated Signal Is an Essential Component of MyD88-Dependent Innate Response to *Mycobacterium tuberculosis* Infection. *The Journal of Immunology*, 179(2), 1178–1189. <https://doi.org/10.4049/jimmunol.179.2.1178>
- Gideon, H. P., Phuah, J., Myers, A. J., Bryson, B. D., Rodgers, M. A., Coleman, M. T., Maiello, P., Rutledge, T., Marino, S., Fortune, S. M., Kirschner, D. E., Lin, P. L., & Flynn, J. L. (2015). Variability in Tuberculosis Granuloma T Cell Responses Exists, but a Balance of Pro- and Anti-inflammatory Cytokines Is Associated with Sterilization. *PLOS Pathogens*, 11(1), e1004603. <https://doi.org/10.1371/journal.ppat.1004603>
- Global Tuberculosis Report. (2024). <https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2024>
- Gopal, R., Monin, L., Torres, D., Slight, S., Mehra, S., McKenna, K. C., Fallert Junecko, B. A., Reinhart, T. A., Kolls, J., Báez-Saldaña, R., Cruz-Lagunas, A., Rodríguez-Reyna, T. S., Kumar, N. P., Tessier, P., Roth, J., Selman, M., Becerril-Villanueva, E., Baquera-Heredia, J., Cumming, B., ... Khader, S. A. (2013). S100A8/A9 Proteins Mediate Neutrophilic Inflammation and Lung Pathology during Tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 188(9), 1137–1146. <https://doi.org/10.1164/rccm.201304-0803OC>
- Griffith, J. W., Sokol, C. L., & Luster, A. D. (2014). Chemokines and Chemokine Receptors: Positioning Cells for Host Defense and Immunity. *Annual Review of Immunology*, 32(1), 659–702. <https://doi.org/10.1146/annurev-immunol-032713-120145>

- Guler, R., Ozturk, M., Sabeel, S., Motaung, B., Parihar, S. P., Thienemann, F., & Brombacher, F. (2021). Targeting Molecular Inflammatory Pathways in Granuloma as Host-Directed Therapies for Tuberculosis. *Frontiers in Immunology*, 12, 733853. <https://doi.org/10.3389/fimmu.2021.733853>
- Harris, J., & Keane, J. (2010). How tumour necrosis factor blockers interfere with tuberculosis immunity. *Clinical and Experimental Immunology*, 161(1), 1–9. <https://doi.org/10.1111/j.1365-2249.2010.04146.x>
- Hmama, Z., Peña-Díaz, S., Joseph, S., & Av-Gay, Y. (2015). Immuno-evasion and immunosuppression of the macrophage by *Mycobacterium tuberculosis*. *Immunological Reviews*, 264(1), 220–232. <https://doi.org/10.1111/imr.12268>
- Keane, J., Gershon, S., Wise, R. P., Mirabile-Levens, E., Kasznica, J., Schwieterman, W. D., Siegel, J. N., & Braun, M. M. (2001). Tuberculosis Associated with Infliximab, a Tumor Necrosis Factor α -Neutralizing Agent. *New England Journal of Medicine*, 345(15), 1098–1104. <https://doi.org/10.1056/NEJMoa011110>
- Khader, S. A., Bell, G. K., Pearl, J. E., Fountain, J. J., Rangel-Moreno, J., Cilley, G. E., Shen, F., Eaton, S. M., Gaffen, S. L., Swain, S. L., Locksley, R. M., Haynes, L., Randall, T. D., & Cooper, A. M. (2007). IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nature Immunology*, 8(4), 369–377. <https://doi.org/10.1038/ni1449>
- Kleinnijenhuis, J., Oosting, M., Joosten, L. A. B., Netea, M. G., & Van Crevel, R. (2011). Innate Immune Recognition of *Mycobacterium tuberculosis*. *Clinical and Developmental Immunology*, 2011, 1–12. <https://doi.org/10.1155/2011/405310>
- Kuroda, M. J., Sugimoto, C., Cai, Y., Merino, K. M., Mehra, S., Araújo, M., Roy, C. J., Midkiff, C. C., Alvarez, X., Didier, E. S., & Kaushal, D. (2018). High Turnover of Tissue Macrophages Contributes to Tuberculosis Reactivation in Simian Immunodeficiency Virus-Infected Rhesus Macaques. *The Journal of Infectious Diseases*, 217(12), 1865–1874. <https://doi.org/10.1093/infdis/jix625>
- Lyadova, I. V., & Panteleev, A. V. (2015). Th1 and Th17 Cells in Tuberculosis: Protection, Pathology, and Biomarkers. *Mediators of Inflammation*, 2015(1), 854507. <https://doi.org/10.1155/2015/854507>
- Marino, S., Cilfone, N. A., Mattila, J. T., Linderman, J. J., Flynn, J. L., & Kirschner, D. E. (2015). Macrophage Polarization Drives Granuloma Outcome during *Mycobacterium tuberculosis* Infection. *Infection and Immunity*, 83(1), 324–338. <https://doi.org/10.1128/IAI.02494-14>
- Mayer-Barber, K., Barber, D., Shenderov, K., White, S., Wilson, M., Cheever, A., Kugler, D., Sutterwala, F., & Sher, A. (2010). Caspase-1 independent IL-1 β Production is critical for MyD88-mediated host resistance to *Mycobacterium tuberculosis* (42.13). *The Journal of Immunology*, 184(Supplement_1), 42.13–42.13. <https://doi.org/10.4049/jimmunol.184.Supp.42.13>
- Mayer-Barber, K. D., Andrade, B. B., Barber, D. L., Hieny, S., Feng, C. G., Caspar, P., Oland, S., Gordon, S., & Sher, A. (2011). Innate and Adaptive Interferons Suppress IL-1 α and IL-1 β Production by Distinct Pulmonary Myeloid Subsets during *Mycobacterium tuberculosis* Infection. *Immunity*, 35(6), 1023–1034. <https://doi.org/10.1016/j.immuni.2011.12.002>
- McGeachy, M. J., Cua, D. J., & Gaffen, S. L. (2019). The IL-17 Family of Cytokines in Health and Disease. *Immunity*, 50(4), 892–906. <https://doi.org/10.1016/j.immuni.2019.03.021>
- Meintjes, G., Lawn, S. D., Scano, F., Maartens, G., French, M. A., Worodria, W., Elliott, J. H., Murdoch, D., Wilkinson, R. J., Seyler, C., John, L., Van Der Loeff, M. S., Reiss, P., Lynen, L., Janoff, E. N., Gilks, C., & Colebunders, R. (2008). Tuberculosis-associated immune reconstitution inflammatory syndrome: Case definitions for use in resource-limited settings. *The Lancet Infectious Diseases*, 8(8), 516–523. [https://doi.org/10.1016/S1473-3099\(08\)70184-1](https://doi.org/10.1016/S1473-3099(08)70184-1)
- Mishra, B. B., Rathinam, V. A. K., Martens, G. W., Martinot, A. J., Kornfeld, H., Fitzgerald, K. A., & Sasseti, C. M. (2013). Nitric oxide controls the immunopathology of tuberculosis by inhibiting NLRP3 inflammasome-dependent processing of IL-1 β . *Nature Immunology*, 14(1), 52–60. <https://doi.org/10.1038/ni.2474>

- Moore, K. W., De Waal Malefyt, R., Coffman, R. L., & O'Garra, A. (2001). Interleukin-10 and the Interleukin-10 Receptor. *Annual Review of Immunology*, 19(1), 683–765. <https://doi.org/10.1146/annurev.immunol.19.1.683>
- Mundra, A., Yegiazaryan, A., Karsian, H., Alsaigh, D., Bonavida, V., Frame, M., May, N., Gargaloyan, A., Abnousian, A., & Venketaraman, V. (2023). Pathogenicity of Type I Interferons in Mycobacterium tuberculosis. *International Journal of Molecular Sciences*, 24(4), 3919. <https://doi.org/10.3390/ijms24043919>
- Nouailles, G., Dorhoi, A., Koch, M., Zerrahn, J., Weiner, J., Faé, K. C., Arrey, F., Kuhlmann, S., Bandermann, S., Loewe, D., Mollenkopf, H.-J., Vogelzang, A., Meyer-Schwesinger, C., Mittrücker, H.-W., McEwen, G., & Kaufmann, S. H. E. (2014). CXCL5-secreting pulmonary epithelial cells drive destructive neutrophilic inflammation in tuberculosis. *Journal of Clinical Investigation*, 124(3), 1268–1282. <https://doi.org/10.1172/JCI72030>
- Opal, S. M., & DePalo, V. A. (2000). Anti-inflammatory cytokines. *Chest*, 117(4), 1162–1172. <https://doi.org/10.1378/chest.117.4.1162>
- Ottenhoff, T. H. M., Kumararatne, D., & Casanova, J.-L. (1998). Novel human immunodeficiencies reveal the essential role of type-1 cytokines in immunity to intracellular bacteria. *Immunology Today*, 19(11), 491–494. [https://doi.org/10.1016/S0167-5699\(98\)01321-8](https://doi.org/10.1016/S0167-5699(98)01321-8)
- Pandiarajan, A. N., Kumar, N. P., Selvaraj, N., Ahamed, S. F., Viswanathan, V., Thiruvengadam, K., Hissar, S., Shanmugam, S., Bethunaickan, R., Nott, S., Kornfeld, H., & Babu, S. (2024). Distinct TB-antigen stimulated cytokine profiles as predictive biomarkers for unfavorable treatment outcomes in pulmonary tuberculosis. *Frontiers in Immunology*, 15, 1392256. <https://doi.org/10.3389/fimmu.2024.1392256>
- Parameswaran, N., & Patial, S. (2010). Tumor necrosis factor- α signaling in macrophages. *Critical ReviewsTM in Eukaryotic Gene Expression*, 20(2). <https://doi.org/10.1615/CritRevEukarGeneExpr.v20.i2.10>
- Philips, J. A., & Ernst, J. D. (2012). Tuberculosis Pathogenesis and Immunity. *Annual Review of Pathology: Mechanisms of Disease*, 7(1), 353–384. <https://doi.org/10.1146/annurev-pathol-011811-132458>
- Ramakrishnan, L. (2012). Revisiting the role of the granuloma in tuberculosis. *Nature Reviews Immunology*, 12(5), 352–366. <https://doi.org/10.1038/nri3211>
- Redford, P. S., Murray, P. J., & O'Garra, A. (2011). The role of IL-10 in immune regulation during M. tuberculosis infection. *Mucosal Immunology*, 4(3), 261–270. <https://doi.org/10.1038/mi.2011.7>
- Roach, D. R., Bean, A. G. D., Demangel, C., France, M. P., Briscoe, H., & Britton, W. J. (2002). TNF Regulates Chemokine Induction Essential for Cell Recruitment, Granuloma Formation, and Clearance of Mycobacterial Infection. *The Journal of Immunology*, 168(9), 4620–4627. <https://doi.org/10.4049/jimmunol.168.9.4620>
- Sampath, P., Rajamanickam, A., Thiruvengadam, K., Natarajan, A. P., Hissar, S., Dhanapal, M., Thangavelu, B., Jayabal, L., Ramesh, P. M., Ranganathan, U. D., Babu, S., & Bethunaickan, R. (2023). Cytokine upsurge among drug-resistant tuberculosis endorse the signatures of hyper inflammation and disease severity. *Scientific Reports*, 13(1), 785. <https://doi.org/10.1038/s41598-023-27895-8>
- Saunders, B. M., Frank, A. A., Orme, I. M., & Cooper, A. M. (2000). Interleukin-6 induces early gamma interferon production in the infected lung but is not required for generation of specific immunity to Mycobacterium tuberculosis infection. *Infection and Immunity*, 68(6), 3322–3326. <https://doi.org/10.1128/iai.68.6.3322-3326.2000>
- Schroder, K., Hertzog, P. J., Ravasi, T., & Hume, D. A. (2004). Interferon- γ : An overview of signals, mechanisms and functions. *Journal of Leukocyte Biology*, 75(2), 163–189. <https://doi.org/10.1189/jlb.0603252>
- Schurz, H., Naranbhai, V., Yates, T. A., Gilchrist, J. J., Parks, T., Dodd, P. J., Möller, M., Hoal, E. G., Morris, A. P., Hill, A. V., & International Tuberculosis Host Genetics Consortium. (2024). Multi-ancestry meta-analysis of host genetic susceptibility to tuberculosis identifies shared genetic architecture. *eLife*, 13, e84394. <https://doi.org/10.7554/eLife.84394>

- Sendide, K., Deghmane, A.-E., Pechkovsky, D., Av-Gay, Y., Talal, A., & Hmama, Z. (2005). Mycobacterium bovis BCG Attenuates Surface Expression of Mature Class II Molecules through IL-10-Dependent Inhibition of Cathepsin S. *The Journal of Immunology*, 175(8), 5324–5332. <https://doi.org/10.4049/jimmunol.175.8.5324>
- Seung, K. J., Keshavjee, S., & Rich, M. L. (2015). Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. *Cold Spring Harbor Perspectives in Medicine*, 5(9), a017863. <https://doi.org/10.1101/cshperspect.a017863>
- Sia, J. K., Georgieva, M., & Rengarajan, J. (2015). Innate Immune Defenses in Human Tuberculosis: An Overview of the Interactions between Mycobacterium tuberculosis and Innate Immune Cells. *Journal of Immunology Research*, 2015, 1–12. <https://doi.org/10.1155/2015/747543>
- Singhal, A., Jie, L., Kumar, P., Hong, G. S., Leow, M. K.-S., Paleja, B., Tsenova, L., Kurepina, N., Chen, J., Zolezzi, F., Kreiswirth, B., Poidinger, M., Chee, C., Kaplan, G., Wang, Y. T., & De Libero, G. (2014). Metformin as adjunct antituberculosis therapy. *Science Translational Medicine*, 6(263). <https://doi.org/10.1126/scitranslmed.3009885>
- Stanley, S. A., & Cox, J. S. (2013). Host–Pathogen Interactions During Mycobacterium tuberculosis infections. In J. Pieters & J. D. McKinney (Eds), *Pathogenesis of Mycobacterium tuberculosis and its Interaction with the Host Organism* (Vol. 374, pp. 211–241). Springer Berlin Heidelberg. https://doi.org/10.1007/82_2013_332
- Toossi, Z., Gogate, P., Shiratsuchi, H., Young, T., & Ellner, J. J. (1995). Enhanced production of TGF- β by blood monocytes from patients with active tuberculosis and presence of TGF- β in tuberculous granulomatous lung lesions. *The Journal of Immunology*, 154(1), 465–473. <https://doi.org/10.4049/jimmunol.154.1.465>
- Torrado, E., & Cooper, A. M. (2010). IL-17 and Th17 cells in tuberculosis. *Cytokine & Growth Factor Reviews*, 21(6), 455–462. <https://doi.org/10.1016/j.cytogfr.2010.10.004>
- Trinchieri, G. (2003). Interleukin-12 and the regulation of innate resistance and adaptive immunity. *Nature Reviews Immunology*, 3(2), 133–146. <https://doi.org/10.1038/nri1001>
- Turner, M. D., Nedjai, B., Hurst, T., & Pennington, D. J. (2014). Cytokines and chemokines: At the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, 1843(11), 2563–2582. <https://doi.org/https://doi.org/10.1016/j.bbamcr.2014.05.014>
- Wallis, R. S., Maeurer, M., Mwaba, P., Chakaya, J., Rustonjee, R., Migliori, G. B., Marais, B., Schito, M., Churchyard, G., Swaminathan, S., Hoelscher, M., & Zumla, A. (2016). Tuberculosis—Advances in development of new drugs, treatment regimens, host-directed therapies, and biomarkers. *The Lancet Infectious Diseases*, 16(4), e34–e46. [https://doi.org/10.1016/S1473-3099\(16\)00070-0](https://doi.org/10.1016/S1473-3099(16)00070-0)
- Walzl, G., McNerney, R., Du Plessis, N., Bates, M., McHugh, T. D., Chegou, N. N., & Zumla, A. (2018). Tuberculosis: Advances and challenges in development of new diagnostics and biomarkers. *The Lancet Infectious Diseases*, 18(7), e199–e210. [https://doi.org/10.1016/S1473-3099\(18\)30111-7](https://doi.org/10.1016/S1473-3099(18)30111-7)
- Weiss, G., & Schaible, U. E. (2015). Macrophage defense mechanisms against intracellular bacteria. *Immunological Reviews*, 264(1), 182–203. <https://doi.org/10.1111/imr.12266>
- Zhang, F., Zhang, F., Dong, Y., Li, L., & Pang, Y. (2023). New Insights into Biomarkers for Evaluating Therapy Efficacy in Pulmonary Tuberculosis: A Narrative Review. *Infectious Diseases and Therapy*, 12(12), 2665–2689. <https://doi.org/10.1007/s40121-023-00887-x>
- Zhuang, L., Yang, L., Li, L., Ye, Z., & Gong, W. (2024). Mycobacterium tuberculosis: Immune response, biomarkers, and therapeutic intervention. *MedComm*, 5(1), e419. <https://doi.org/https://doi.org/10.1002/mco2.419>
- Zumla, A., Rao, M., Wallis, R. S., Kaufmann, S. H., Rustonjee, R., Mwaba, P., Vilaplana, C., Yeboah-Manu, D., Chakaya, J., & Ippolito, G. (2016). Host-directed therapies for infectious diseases: Current status, recent progress, and future prospects. *The Lancet Infectious Diseases*, 16(4), e47–e63. [https://doi.org/10.1016/S1473-3099\(16\)00078-5](https://doi.org/10.1016/S1473-3099(16)00078-5)



Synthetic Morphogen Gradient Engineering in Human Organoids: Toward Programmable Tissue Patterning for Next-Generation Regenerative Medicine

Esoh Rene Tanwiah ^{1,2*} , Esoh Stephano Bezeng ³, Kiranjot Kaur ¹ , John Ricky ⁴, Simon Boateng ⁵, Rajshreejeet Jayant Bhensdadiya ⁶, Solomon Gyampoh ⁷, and Shey Fortune Ndzi ⁸ 

¹ Department of Biotechnology and Food Technology, Punjabi University, Patiala, Punjab, India.

² Health Organisation Welfare (HOW), Yaoundé, Cameroon.

³ Department of Computer Science and Engineering, Bahra University, Wagnaghat, Himachal Pradesh (HP), India.

⁴ Department of Biomedical Engineering, Kocaeli University, Umuttepe Campus, 41380 İzmit, Kocaeli, Türkiye.

⁵ The Catholic University of America School of Engineering, USA.

⁶ Department of Pharmacology, Government Medical College, Bhavnagar, Gujarat, India.

⁷ Chitkara College of Pharmacy, Chitkara University, Punjab, India.

⁸ Florence Nightingale School of Health and Biomedical Sciences, Bamenda, Cameroon.

ARTICLE INFO

Received: 29th May 2026

Received in revised form: 08th July 2026

Accepted: 12th July 2026

Published: 15th September 2026

*Corresponding Author:

Esoh Rene Tanwiah

E-mail :

esohrene999@gmail.com

Citation : Tanwiah, E. R., Bezeng, E. S., Kaur, K., Ricky, J., Boateng, S., Bhensdadiya, R. J., Gyampoh, S., & Ndzi, S. F. (2026). Synthetic Morphogen Gradient Engineering in Human Organoids: Toward Programmable Tissue Patterning for Next-Generation Regenerative Medicine. *Modern Journal of Health and Applied Sciences*, 3(2), 61-90.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

DOI: <https://doi.org/10.70411/MJHAS.3.2.2026408>

ABSTRACT

Despite impressive self-organisation, conventional human organoids (cell aggregates) fail to reproduce intricate, reproducible, and scalable in vivo-like structures and organ formations because they rely on uniform (constant) pro-differentiation morphogen gradients. To address this developmental impasse, the field has evolved toward synthetic gradient engineering with morphogen signals, which combines developmental signalling cues with cutting-edge synthetic biology and bioengineering platforms. Here, we present a comprehensive overview of cutting-edge advances in four main areas: novel gradient engineering systems (ranging from microfluidic chips to sustained-release hydrogels, optogenetics, and DNA-barcoded microbeads); the types of organoids that respond to these spatial cues; the engineered "Morphogenic Programming" circuits that allow cells to automatically interpret morphogen thresholds and record positional history; and the profound translational implications of these advances. The growing body of evidence across these platforms suggests an interesting convergence: tissue patterning is largely achieved through hybrid approaches that combine external spatial cues with intrinsic self-organisation. Synthetic gradient engineering provides the means to transform cell aggregates into customised structures, enabling spatially resolved drug screening, reliable modelling of monogenic diseases, and novel therapeutic strategies for tissue regeneration.

Keywords: Morphogen gradients, Organoids, Synthetic biology, Gene circuits, Tissue engineering, Regenerative medicine.

1. Introduction

1. 1. A Developmental Paradox and a Technological Gap

A long-standing paradox in developmental biology is how a seemingly uniform blastula yields hundreds of distinct cell types arranged into reproducible anatomical structures, largely guided by noisy, diffusing morphogen signals. Turing's reaction-diffusion theory proposed that patterns could arise *de novo* from instabilities between short-range activators and long-range inhibitors, whereas Wolpert's positional information framework emphasised graded morphogen concentrations that cells interpret as coordinates (Turing, 1952; Wolpert, 1969). Contemporary studies of neural tube, limb, and gut development have confirmed the positional information framework originally proposed by Wolpert (1969). We now know that this process is driven by specific families of morphogens, especially Wnt, Bone Morphogenetic Protein (BMP), Sonic hedgehog (Shh), Fibroblast Growth Factor (FGF), and retinoic acid (RA), which form spatial and temporal gradients to assign positional identity (Green & Sharpe, 2015; O'Grady et al., 2019).

Organoid technology offers three-dimensional human stem cell models that recreate developmental processes. These systems can develop structures resembling the brain, intestines, kidneys, lungs, liver, and heart. They often grow from unpatterned stem cell aggregates exposed to simple, time-based sequences of morphogen addition and removal, mirroring natural embryo formation. However, while their ability to self-organise is impressive, standard organoids usually fail to develop the precise, repeatable patterns and large-scale architecture found in the human body. For example, cortical layers may be scattered, gut structures may be incomplete, and heart chambers may be inconsistent across different batches. To address this, researchers have designed environments that provide multiple signalling gradients, mechanical cues, and long-range interactions, replacing the uniform liquid media traditionally used in standard cultures.

1. 2. The Problem of Controlled Tissue Patterning in Organoids

It is becoming apparent that human organoids can produce complex cell fate diversity in response to modified regimens of morphogens or their antagonists. However, the proportion and configuration of cell fates vary between cell lines and even between aliquots of the same cell line (Anand et al., 2023; Sanchís-Calleja et al., 2026; Scuderi et al., 2025). For instance, single-cell RNA-seq-based morphogen screening in neural organoids has shown that gradient exposure to Shh, Wnt, FGF8, RA, or BMP modulators dramatically alters the proportions of dorsal-ventral and anterior-posterior neurones but also shows a significant inter-line variability in morphogen responses and patterning (Sanchís-Calleja et al., 2026). Likewise, microfluidic tools that deliver opposing gradients of Wnt agonists and antagonists, or gradient exposure to orthogonal Wnt and Shh agonists, to endodermal or neural spheroids can achieve spectacular anterior-posterior and dorsal-ventral patterning of endodermal or neuronal tissues. However, patterning outcomes vary between genetic models and even within cultures (O'Grady et al., 2019; Scuderi et al., 2025; Yaman & Ramanathan, 2023). These findings suggest two related issues. First, external morphogen presentation must be much better controlled in space and time than simple bath application (especially when multiple pathways need to be altered), which can be achieved by simple bath application (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023). Second, the gene regulatory networks that respond to these gradients are not static; without engineered mechanisms to bring the proposed gradients to bear on network thresholds, memory, and noise, even clear-cut external gradients may not translate into robust tissue-scale patterns (Dupin et al., 2022; Prochazka et al., 2022; Trentesaux et al., 2023). Therefore, a major challenge remains in turning organoids, which only roughly self-organise under gross morphogen manipulations, into engineered platforms for programmable tissue-scale organisation at reproducible scales across donors and batches.

1. 3. Modern Gradient Biology: From Reaction Diffusion to Positional Information

Contemporary gradient biology merges the ideas of Turing and Wolpert to provide a more sophisticated view in which multiple contact-dependent and diffusible information sources cooperate to expand and deform tissues (**Figure 2**) (Turing, 1952; Wolpert, 1969). Reaction-diffusion can produce primary axes or spotted and striped patterns, whereas positional information often sharpens such founder asymmetries into sharp boundaries through threshold gene expression and cross-repressive transcription factor wiring (Green & Sharpe, 2015; Wolpert, 1969). In experiments on the vertebrate neural tube, for instance, the neural tube interprets opposing gradients of Shh (floor plate) and BMP/Wnt (roof plate) via cross-regulatory TFs to produce distinct progenitor regions (Green & Sharpe, 2015; Wolpert, 1969). Similarly, in the gut, Wnt activity is high at the base of the crypts, while BMP/Notch gradients regulate stem cell activity and progenitors along the crypt-villus axis (Xiang et al., 2024; Xu et al., 2025). The interpretation of gradients is more than a mere concentration reading. While historical theories have emphasised the importance of positional information and reaction-diffusion (Turing, 1952; Wolpert, 1969), contemporary experimental systems have highlighted the importance of mechanical restraints (O'Grady et al., 2019) and tissue-scale feedback and growth (Green & Sharpe, 2015) in shaping effective morphogen landscapes.

This shift in the understanding of development has two lessons for organoid engineering. First, the simple mimicry of a single snapshot of the concentration profile of a particular morphogen is unlikely to recapitulate in vivo developmental outcomes; instead, dynamic landscapes with complex morphogen interactions need to be designed, often in conjunction with mechanical and matrix interactions (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Zhang et al., 2026). Second, to achieve embryo-like quality control, programmed gradients may need to be complemented by engineered intracellular networks that implement robust state switching mechanisms, including bistability, oscillations, and epigenetic memory (Barkai & Shilo, 2009; Gierer & Meinhardt, 1972; Prochazka et al., 2022). Why Current Organoid Protocols Fail to Recapitulate Authentic Tissue Architecture

Several converging lines of evidence suggest that standard organoid protocols struggle to reproduce authentic tissue architectures. The uniform application of high concentrations of morphogens or their inhibitors often drives organ-wide adoption of a particular cell fate, abolishing regional diversity and collapsing gradients that should exist at the scale of hundreds of micrometres (Sanchís-Calleja et al., 2026; Xiang et al., 2024; Xu et al., 2025). Even when morphogens are pulsed or globally titrated, differences in diffusion across Matrigel (a 3D cell-culture matrix), local cell density, and endogenous ligand production create uncontrolled microgradients that vary between wells and over time, contributing to stochastic organization (Ahmed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). In addition, important structures in vivo, such as signalling zones and organisers, result from spatiotemporal, localised production of morphogens, which are difficult to achieve using bulk medium manipulations (Green & Sharpe, 2015; Marti-Figueroa & Ashton, 2017).

Another limitation is the biophysical environment. Many organoids develop in spherical geometries that lack clear boundaries or axes; therefore, even when external gradients are imposed, they may be misaligned with intrinsic symmetry-breaking events (Yaman & Ramanathan, 2023). Limited vascularisation leads to hypoxia and nutrient gradients that can either confound or override the intended morphogen patterns, particularly in larger organoids (Ahmed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Finally, the absence of stromal, immune, and vascular compartments removes important sources and sinks of morphogens and antagonists that dynamically sculpt gradients in vivo (Ahmed & Kalangi, 2024; Yao et al., 2024). Collectively, these factors mean that current protocols often succeed in producing the right “parts list” of cell types but fail to achieve the correct spatial arrangement or organ-scale geometry.

1. 4. The Synthetic Biology Opportunity: Engineering Programmable Gradient Systems

As an alternative to modifying the external culture environment, synthetic biology provides powerful tools for genetically programming cells. Scientists can now design cells that respond predictably to specific morphogen thresholds, introduce entirely synthetic signalling pathways that function alongside natural ones without interference, and equip cells with genetic memory to

track and respond to their spatial history (Prochazka et al., 2022; Pulecio et al., 2017; Shi et al., 2025; Trentesaux et al., 2023). Synthetic morphogen systems in microbial and cell-free droplets have demonstrated that relatively simple circuits, such as the mutual inhibition of transcription factors or two-node feedback modules, can extract positional information from gradients and form stable expression domains, even in the presence of noise (Dupin et al., 2022; Lam et al., 2022; Schaerli et al., 2014). Recent work on human pluripotent stem cells has used optogenetic activation of Wnt signalling in spatially restricted subpopulations to drive self-organisation and epithelial mesenchymal transition dependent patterning, illustrating that engineered signalling asymmetries can trigger tissue-scale morphogenesis (Repina et al., 2023).

Concurrently, microfluidic, hydrogel, and nanoengineered DNA bead platforms now allow externally imposed gradients with single-organ and even intra-organoid resolution (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Zhang et al., 2026). Organoid development is a revolutionary process. Beyond leveraging global cues to orchestrate simple self-organisation, organoids are now being viewed as engineerable materials. Both spatiotemporal environments and genetic networks that read them can be tailored. Critically, we observe that tuning the external environment and the internal network together, not in isolation, significantly improves tissue patterning fidelity and reliability (Dupin et al., 2022; Prochazka et al., 2022; Repina et al., 2023; Trentesaux et al., 2023).

1. 5. Aim and Outline of this Review

This review draws on research in the fields of developmental gradient biology, organoid engineering, and synthetic biology to develop a new paradigm for synthetic morphogen gradient engineering in human organoids (Figure 1). It describes a conceptual framework and reviews four key areas: (i) gradient engineering platforms, (ii) gradient-patterned organoid systems, (iii) molecular circuits for gradient computation, and (iv) translational considerations in regenerative medicine and drug discovery. It further provides a critical integration of consensus and controversy; identifies less-studied dimensions such as interactions between gradient signaling and the immune system and stroma; and explores research, clinical, and conceptual advances. The focus is on human organoid systems and programmable patterning versus a comprehensive review of all organoid types or animal systems.

To synthesise the current state of synthetic morphogen gradient engineering, a comprehensive literature search was conducted across major electronic databases, including PubMed, Web of Science, Scopus and Google Scholar, with an emphasis on peer-reviewed articles published through late 2025. The search strategy utilized Boolean combinations of core concepts, including "organoid", "morphogen gradient", "synthetic biology", "optogenetics", and "microfluidics". Article selection prioritised foundational theoretical frameworks alongside modern primary research, and reviews focused specifically on programmable patterning in human pluripotent or adult stem cell-derived organoid systems. To maintain a rigorous focus on validated, human-relevant applications, studies investigating invertebrate or plant models were intentionally excluded, along with non-peer-reviewed preprints and conference proceedings. Ultimately, rather than providing an exhaustive catalogue of all synthetic biology research, the retrieved literature was critically appraised and synthesized into four thematic domains: gradient engineering platforms, gradient-patterned organoid systems, molecular circuits for gradient computation, and translational implications for regenerative medicine.

2. Conceptual Framework

2. 1. Core Morphogen-Mediated Signaling in Patterning

During development, core morphogens, such as Wnt/ β -catenin, BMP, Shh, FGF, Notch, and RA, are expressed in complex, overlapping concentration gradients, which are translated into patterning and boundary restriction cues by cells to propagate tissue axes and borders. These evolutionarily conserved signaling pathways are gaining momentum as control levers of choice to direct spatial patterning and tissue regionalization in engineered organoids. Table 1 provides an overview of these key morphogens, their roles in development, and their use in engineered organoid systems.

2. 2. Organoid Self-Organisation: Successes and Randomness

During the last decade, organoids have convincingly shown that human stem cells are endowed with strong self-organising powers. Brain organoids produce primitive regions of cortical layering, ventricular zones, and subtype-specific neurones; gut organoids produce crypt-villus-like structures; kidney organoids produce glomerulus- and tubular-like structures; and liver, lung, and heart organoids recapitulate elements of organ-specific physiology (Ahmed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025; Xiang et al., 2024; Xu et al., 2025; Yao et al., 2024). These structures arise from initially random aggregates through relatively straightforward protocols of morphogen exposure and removal, a process reminiscent of natural development (Ahmed & Kalangi, 2024; Wang et al., 2025). However, organoid self-organisation is unpredictable. Experimental studies comparing the outcomes of different human-induced pluripotent stem cell (iPSC) lines have shown considerable variability in sensitivity to morphogens and the resulting tissue composition, even when using the same protocol (Anand et al., 2023; Sanchís-Calleja et al., 2026; Scuderi et al., 2025).

The timing and location at which these cell aggregates initiate the formation of structure, such as polarisation, lumen formation, or bud production, occur in a stochastic manner. As a result, organoid structures assume variable shapes and irregular internal structures (Figure 3) (Anand et al., 2023; Yaman & Ramanathan, 2023). Furthermore, the inherent production of Wnt, BMP, and other ligands by differentiating cells provides dynamic sources of poorly understood gradients that vary among organoids (Ahmed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; Sanchís-Calleja et al., 2026). These are crucial for studying the properties of developmental robustness but are less desirable for organoids used in clinical applications.

2. 3. Principles of Synthetic Biology Design for Gradient Engineering

Synthetic biology enables a design-driven engineering approach to organoid systems based on the concepts of modularity and orthogonality. First, it is possible to create new independent ('orthogonal') signaling networks based on engineered receptor-ligand pairs or novel transcriptional regulatory systems. This modular approach enables researchers to establish synthetic gradients of morphogens. Surrogate signals, such as secreted fluorescent proteins or synthetic peptides, are used because they do not cross-talk with native signaling pathways (Prochazka et al., 2022; Trentesaux et al., 2023). Second, nonlinear input-output: bistable switches, ultrasensitive promoters, and mutual inhibition can sharpen external, smooth gradients, sharpening domain transitions to improve sharpness and boundary detection (Dupin et al., 2022; Lam et al., 2022; Schaerli et al., 2014). Third, feedback and feedforward: positive feedback can help commit cell fates once a threshold is passed, whereas negative feedback and incoherent feedforward can act as noise minimization or filtering to modulate dynamic responses (Dupin et al., 2022; Lam et al., 2022). Fourth, memory and recording: CRISPR-based epigenetic editing and recording systems can maintain a memory of past morphogen exposure, allowing cells to respond to a time-averaged history (Pulecio et al., 2017; Shi et al., 2025).

For organoids, these ideas point to a design strategy in which control over external gradients (engineered via microfluidics, hydrogels, and optogenetics) is paired with control over the transfer function inside cells, which together dictate tissue patterns. Remarkably, we found that genetic circuits designed in microbes or two-dimensional (2D) cultures often preserve their qualitative behaviour in three-dimensional (3D) organoid contexts but need to be "retuned" in terms of thresholds, response times, and integration with endogenous processes (Lam et al., 2022; Prochazka et al., 2022; Trentesaux et al., 2023).

2. 4. Gradient Systems in Organoids

For theoretical purposes, it is helpful to conceptualise three types of gradient systems in organoid patterning (Figure 2). Endogenous gradients self-organise via intrinsic morphogen secretion, diffusion, and decay, such as the intrinsic Wnt and BMP gradients in growing intestinal organoids or polarity-driven axial elongation in human trunk-like organoids (Anand et al., 2023; Xiang et al., 2024; Xu et al., 2025). Externally imposed gradients are provided by engineered devices and materials, such as stable linear gradient devices using simple diffusion, hydrogels with microfluidic channels that deliver opposing morphogens to the organoid, or DNA-containing microbeads that are microinjected into the organoid (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). Hybrid systems exploit engineered gradients to initiate or bias intrinsic self-organization,

which then develops and stabilizes through feedback processes. For example, optogenetic induction of Wnt in a subpopulation of cells, which in turn secretes more morphogens for nearby wild-type cells, can be used (Repina et al., 2023).

In reality, the most promising organoid applications are of this hybrid nature, in which engineered gradients provide initial biases or induce symmetry breaking, allowing intrinsic patterning processes to complete the pattern development. The trade-off between control and beneficial self-organization has been the subject of many contemporary debates and can also be found in the following sections on limitations and prospects.

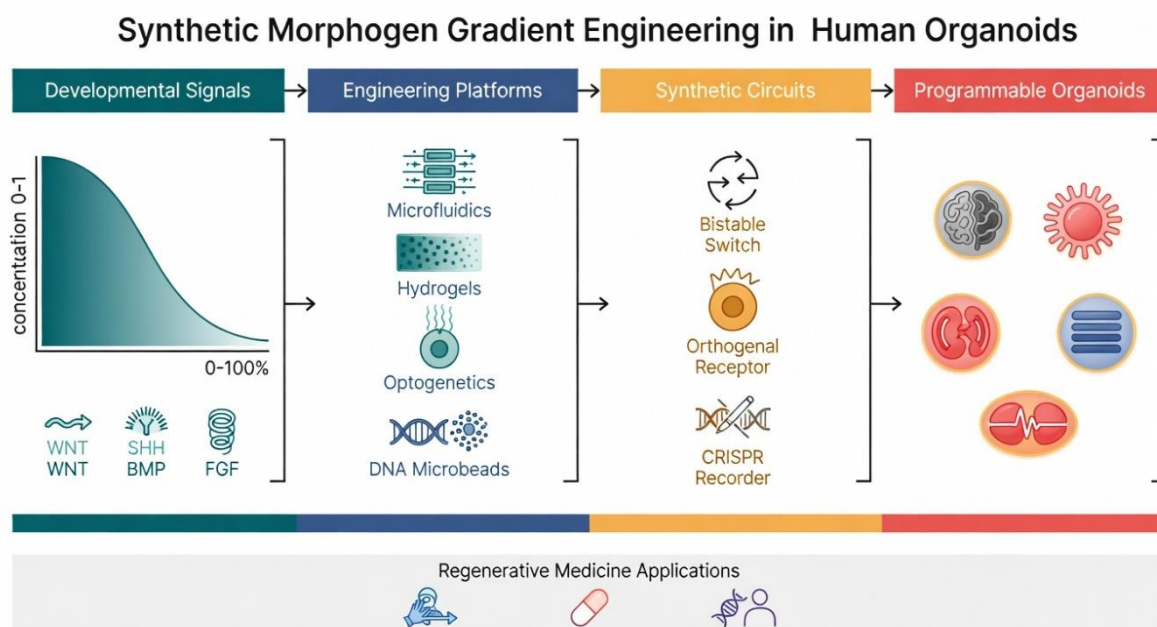


Figure 1. Synthetic engineering framework for human organoids. The spatiotemporal control of morphogen gradients using physical platforms and synthetic circuits enables the generation of programmable organoids with targeted applications in regenerative medicine.

Table 1. Key Morphogen Signalling Pathways and Their Roles in Tissue Patterning Relevant to Organoid Engineering.

Pathway	Representative ligands/molecules	Canonical downstream effects	Developmental/tissue context	Relevance to organoid patterning	Key references
Wnt/β-catenin	Wnt3a, Wnt7a, CHIR99021 (Glycogen Synthase Kinase 3-GSK3 inhibitor)	It stabilizes β -catenin, activates T-cell factor/lymphoid enhancer factor (TCF/LEF) target genes, and promotes proliferation and posterior/ventral fates.	Neural tube anterior-posterior/dorsal-ventral (AP/DV) patterning, intestinal crypt stem cell niche, and early cardiac and kidney specification.	Controls cortical arealization in brain organoids and the crypt-villus axis in intestinal organoids; used in many protocols as a stemness and posteriorizing factor.	(Green & Sharpe, 2015; Repina et al., 2023)
BMP/Transforming Growth Factor Beta (TGFβ)	BMP2/4/7, TGF β 1, Activin A, Noggin (inhibitor).	It phosphorylates Small Mothers Against Decapentaplegic (SMAD)1/5/8 (BMP) or SMAD2/3 (TGF β) and regulates germ layer specification and DV patterning.	Gastrulation and germ layer allocation, dorsal-ventral neural and mesodermal patterning, and intestinal differentiation gradients.	It is widely used to direct ectoderm vs. mesendoderm fates and to modulate villus vs. crypt identity; spatial BMP gradients affect intestinal and lung organoid architecture.	(O'Grady et al., 2019; Xiang et al., 2024)
Sonic hedgehog (Shh)	Shh ligand, Smoothened Agonist (SAG) (agonist), cyclopamine (antagonist).	It Activates Glioma-Associated Oncogene-GLI transcription factors, ventralizes neural tissues, and patterns limb buds.	Ventral neural tube, forebrain, spinal cord, and patterning of motor neuron and interneuron domains.	Local Shh sources or gradients in forebrain organoids specify ventral fates; dual Shh–Wnt gradients generate complex brain regionalization.	(Scuderi et al., 2025; Yao et al., 2024)
FGF	FGF2, FGF4, FGF8, and FGFR agonists were used.	Activates Rat Sarcoma – Mitogen-Activated Protein Kinase (RAS–MAPK) and Phosphoinositide 3-Kinase – Protein Kinase B (PI3K–AKT), promotes proliferation, survival, and patterning at organizer boundaries.	Midbrain–hindbrain organizer; limb outgrowth; kidney and lung branching morphogenesis.	Supports the expansion of progenitors in brain, kidney, and lung organoids; FGF8 gradients help specify neural regional identities when combined with Wnt and RA.	(Green & Sharpe, 2015; Yaman & Ramanathan, 2023)
Notch	Delta-like Canonical Notch Ligand (DLL)1/4, Jagged ligands, and γ -secretase modulator.	Lateral inhibition maintains progenitors and sharpens boundaries by repressing neighbouring cell fates.	Somite segmentation; intestinal crypt stem cell vs secretory fate decisions; vascular sprouting.	Modulates differentiation patterns in intestinal, liver, and vascular organoids; it often acts downstream of primary morphogen gradients to refine architectures.	(Xiang et al., 2024; Xu et al., 2025)

Retinoic acid (RA)	RA, retinaldehyde dehydrogenase (RALDH) enzymes.	Nuclear receptor signalling specifying posterior neural and endodermal identities.	Anterior–posterior patterning of spinal cord, hindbrain, and gut; limb proximodistal patterning.	RA gradients in microfluidic or hydrogel systems drive spinal motor neuron differentiation and axial patterning in neural and endodermal organoids.	(Anand et al., 2023; O’Grady et al., 2019)
EGF family	EGF, heregulin, EGF-like ligands.	Epidermal Growth Factor / Receptor (EGFR)–RAS–MAPK activation promotes proliferation and survival of epithelial lineages.	Intestinal and gastric epithelium; skin and other barrier tissues.	Maintains stem/progenitor compartments in intestinal and gastric organoids; contributes to crypt domain expansion and villus patterning.	(Wang et al., 2025; Xiang et al., 2024)
VEGF	VEGF-A, VEGF-C, VEGF-D.	Drives endothelial proliferation, migration, and tube formation via Vascular Endothelial Growth Factor / Receptor (VEGFR2/3).	Vascular network formation in many organs; tip/stalk cell patterning.	Used in vascularized liver and cardiac organoids, VEGF gradients are potential tools for engineering perfusable vascular domains within organoids.	(Yao et al., 2024; Zhang et al., 2026)

Note. Data synthesised from Green & Sharpe (2015), Xiang et al. (2024), Yao et al. (2024), and O’Grady et al. (2019). AP/DV: Anterior-Posterior / Dorsal-Ventral; BMP: Bone Morphogenetic Protein; CHIR99021: Glycogen Synthase Kinase 3 (GSK3) inhibitor; FGF: Fibroblast Growth Factor; RA: Retinoic Acid; SAG: Smoothened Agonist; Shh: Sonic hedgehog; TGFβ: Transforming Growth Factor Beta, VEGF: Vascular Endothelial Growth Factor.

3. Engineering Synthetic Morphogen Gradients, Platforms, and Technologies

3.1. Microfluidic Gradient Generators

Microfluidic devices remain the most mature platform for engineering defined morphogen gradients in stem cell cultures. While foundational studies in the early 2000s relied on active-flow branched channels for 2D cultures, modern systems utilise passive dual-reservoir diffusion devices to generate stable, pumpless linear gradients across trapped 3D organoids (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). Mathematical models confirm that these devices successfully establish reproducible gradients of key morphogens (e.g., Wnt, Shh, and BMPs) at spatial scales appropriate for human development (Yaman & Ramanathan, 2023). These gradient dynamics are fundamentally governed by the reaction-diffusion equation 1:

$$\frac{\partial C}{\partial t} = D \nabla^2 C - R(C) \quad (1)$$

Here, C represents the morphogen concentration, D is the diffusion coefficient, and $R(C)$ accounts for the reaction or cellular consumption rate. A comprehensive comparison of these microfluidic gradient configurations and their capabilities is detailed in Table 2.

Applied to human neural and endodermal systems, microfluidic gradients have generated countervailing Wnt/inhibitor fields that pattern anterior–posterior and dorsal–ventral axes within organoids and spheroids (Sanchís-Calleja et al., 2026; Scuderi et al., 2025; Yaman & Ramanathan, 2023). Orthogonal-gradient platforms, such as the Dual Microgradient Array Patterning System (Duo-MAPS), expose iPSC aggregates to intersecting gradients of Wnt and Shh agonists, producing organoids containing forebrain, midbrain, and hindbrain-like regions whose transcriptomes map onto foetal brain atlases (Scuderi et al., 2025). Passive diffusion-based gradient generators have also been used to apply combined Wnt, BMP, and RA gradients that drive forebrain-hindbrain and dorsal-ventral patterning in Matrigel-free neural organoids, highlighting the feasibility of scalable, matrix-independent devices (Yaman & Ramanathan, 2023).

Microfluidic systems are powerful in precision and flexibility but have limitations. Chip fabrication and use, while improving, remain technically challenging, and scaling up the number of organoids while ensuring reproducible and consistent flow and gradient profiles between devices remains difficult (Huang et al., 2021; Marti-Figueroa & Ashton, 2017). Further, organoid culture in microfluidic chips can be affected by shear stress and challenging for downstream imaging or analysis, although organoid-on-chip systems increasingly include gentle perfusion and modular retrieval capabilities (Ahmed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025). Critically, what we learn from microfluidics literature is that exogenous gradients can lead to robust biases in organoid patterning, but how to balance them with intrinsic signalling and morphogen production remains challenging and needs to be studied for each system (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Scuderi et al., 2025).

3.2. Slow-Release and Channel Gradients in Hydrogels

Hydrogels offer an alternative approach in which gradients are not imposed upon the gel but rather encoded within by sources and sinks. A notable variant employs channels lithographically inscribed within a thick hydrogel containing stem cells; fluid channels are perfused with different solutions containing morphogens to generate steady gradients by diffusion through the matrix (O'Grady et al., 2019). O'Grady and colleagues showed fluidic-channel hydrogels can establish opposing gradients of RA and Transforming Growth Factor Beta (TGFβ)/BMP to mesenchymal stem cells, resulting in spatially varying differentiation from osteoblasts to chondrocytes in a single patch (O'Grady et al., 2019). Simulations of diffusion constants and population-level consumption rates allow the design of gradient profiles and time windows of exposure; these are tested via reporter cell lines (O'Grady et al., 2019).

More generally, gels containing microparticles or microspheres are able to slowly release morphogens such as BMP2, TGF β 1, or synthetically active Wnt proteins, whose release profile can be influenced by particle type and loading method (Marti-Figueroa & Ashton, 2017; Zhang et al., 2026). Co-delivery of two particles with different degradation times enables step- or spatial patterns of combined exposure to two factors, more closely mimicking natural development (Marti-Figueroa & Ashton, 2017). Although many of these examples have involved studies of mesenchymal or skeletal tissue-like structures, the general principles can be applied to epithelial organoids in extracellular matrix (ECM)-like hydrogels. A recent study of organoids embedded in controlled synthetic materials, including dynamic hydrogels with user-defined stiffness and ligand availability, highlighted the role of multi-scale mechanical and biochemical gradients in organogenesis (Zhang et al., 2026).

Hydrogels are appealing to many researchers because they are macroscopically simple and can be used with standard furnaces and microscopes, making them easier to adapt. But it can be difficult to control gradients at the scale of individual organoids when many aggregates are embedded in a shared gel, and diffusion through complex organoid-matrix geometries can result in unexpected distributions of morphogens (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). In addition, temporal control is generally more difficult than in microfluidic systems, for example, to produce sharp on-off pulses unless combined with an active component such as photolabile linkers and release-on-demand.

3. 3. Optogenetic and Chemogenetic Approaches

A powerful way to achieve precise temporal control over signaling proteins is through optogenetics. In this approach, the effective "morphogen" is not the physical concentration of a molecule but rather the activity level of a light-sensitive signalling pathway. For instance, Repina et al. developed optoWnt in human embryonic stem cells (hESCs). This system uses the clustering of a Wnt pathway component, triggered by blue-light illumination, to produce rapid kinetic control and activation of β -catenin-dependent canonical Wnt signalling. By selectively illuminating different cell populations, researchers spatially patterned Wnt activation in hESC co-cultures. This induced epithelial-mesenchymal transitions and mesendoderm differentiation, ultimately resulting in beautifully self-organised epithelial and mesenchymal domains. Similarly, optogenetic activation has been used in cardiac differentiation to control mesoderm induction and patterning.

In theory, illuminating subregions of a 3D organoid could be used to create arbitrary virtual gradients of pathway activity without the need to expose the tissue to a gradient of a soluble ligand. While most existing examples are from 2D or shallow 3D cultures, which are restricted by the ~100–300 μ m penetration limit of blue light, the adoption of redshifted optogenetic tools takes advantage of the biological optical window. By utilizing red and far-red wavelengths, these tools enable significantly deeper tissue penetration (up to ~1 mm depending on tissue opacity), facilitating intricate spatial and temporal patterning even within larger, maturing organoids (Hellwarth et al., 2021; Repina et al., 2023). Chemogenetic control formats like synthetic receptors triggered by non-native small molecules similarly allow appealing capabilities, particularly when combined with microfluidic or hydrogel-based control of ligand spatial patterning (Prochazka et al., 2022; Trentesaux et al., 2023).

A major advantage of opto- and chemogenetic systems is that they decouple activation of receptors from native ligand kinetics, enabling clear dissection of pathway roles and interactions. However, such approaches require genetic manipulation of stem cells, complex illumination or drug-delivery systems, and careful consideration of potential off-target effects and toxicity (Hellwarth et al., 2021; Repina et al., 2023). To translate such systems, the integration of optogenetic patterning into good manufacturing practice (GMP)-compatible processes will be challenging, but cell pre-patterning early in the process, followed by device-free maturation, remains a possibility.

3. 4. DNA-Based Programmable Morphogen Scaffolds

The emerging "nano-engineered" DNA-based hydrogels and microbeads represent a new type of gradient engineering platform in which the morphogen is linked to a DNA network that can be precisely positioned and activated (Afting et al., 2024). Afting et al. delivered microbeads made of DNA with variable stiffness and surface chemistry into medaka retinal organoids via microinjection to provide localised, light-cleavable sources of a mimic for the Wnt morphogen (Afting et al., 2024). The attachment of the morphogen to the DNA through photocleavable linkers resulted in spatiotemporal release of the morphogen from within the retina, generating inside-out gradients more akin to some in vivo situations where the signalling centre is in the interior of the tissue (Afting et al., 2024). These gradients led to the preferential formation of pigmented epithelium (PigE) at the expense of neuroretinal ganglion cells (NG) in the organoid, thereby enhancing its realism (Afting et al., 2024).

Theoretically, DNA scaffolds could serve as 'smart' scaffolds. By incorporating molecular logic gates, these scaffolds will be able to respond to tissue-specific cues and selectively deliver morphogens, providing dynamic feedback to the state of the tissue (Lam et al., 2022; Schaerli et al., 2014). While such integrated systems remain (mostly) theoretical, the field of DNA nanotechnology has reached a stage where it is possible to program such "smart" scaffolds today. Lastly, translating these technologies into human clinical use may be accompanied by concerns about immunogenicity and degradation; however, pathways may be cleared by existing clinical use of DNA-based therapeutics.

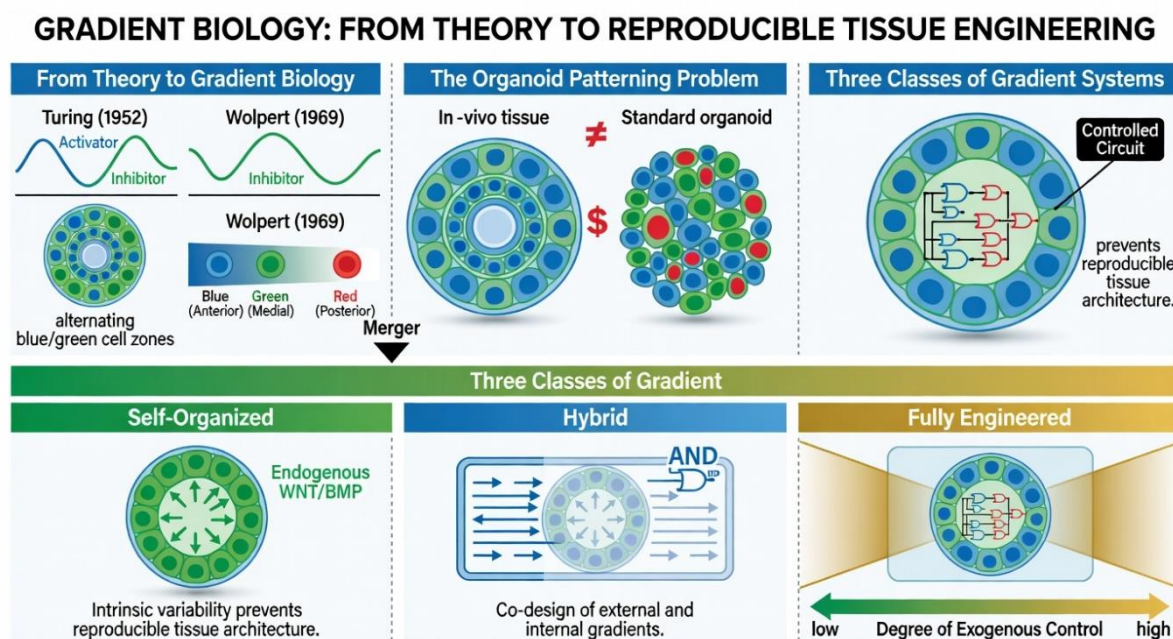


Figure 2. A graphical abstract illustrating the evolution of gradient biology from foundational theories (Turing, 1952; Wolpert, 1969) to modern tissue engineering, categorising approaches into self-organised, hybrid, and fully engineered systems to overcome the organoid patterning problem.

Table 2. Comparison of Synthetic Gradient Engineering Platforms for Organoid Patterning Applications.

Platform type	Mechanism of gradient formation	Spatial resolution	Temporal control	Organoid compatibility	Key limitations	Representative studies
Passive-diffusion microfluidic chips	Morphogens diffuse from side reservoirs into the central organoid channel, establishing steady linear gradients.	Tens to hundreds of micrometres across the channel; shape tunable by geometry and concentrations.	Stable over 24-48 h; gradient can be reconfigured by changing reservoir contents.	Neural and endodermal organoids; embryoid bodies; Matrigel-free systems.	Device fabrication/operation complexity; shear stress; limited throughput if each device holds few organoids.	(Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023)
Orthogonal-gradient microfluidics (Duo-MAPS)	Two perpendicular diffusion axes with distinct morphogens forming orthogonal gradients around spheroids.	Approximate quadrant-level patterning with regional domains on a 100-500 µm scale.	Independent control of each gradient over days via reservoir changes.	Brain organoids and neural spheroids are adaptable to other organoids with size constraints.	Alignment of organoids with axes; variable responses across iPSC lines.	(Scuderi et al., 2025; Yao et al., 2024)
Hydrogel-embedded fluidic channels	Channels patterned inside hydrogels perfused with distinct morphogen-containing media generate diffusion-based gradients.	Length scales from hundreds of micrometres to millimetres; multi-gradient configurations are possible.	Good temporal control via perfusion pumps; reversible by switching channel contents.	Mesenchymal constructs, organoids embedded in gels, multi-tissue assemblies.	Complex fabrication; diffusion through tissue yields non-trivial profiles; limited single-organoid targeting.	(Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019)
Microparticle-based slow-release hydrogels	Morphogens loaded into degradable or mineral-coated microparticles embedded in ECM-like gels create long-lived gradients.	Coarse to moderate; depends on particle size and distribution, typically 100–1000 µm.	Long-term, days to weeks; temporal profiles set by degradation and diffusion.	Bone, cartilage, and potentially epithelial organoids with appropriate ECM.	Less precise spatial boundaries; difficult to rapidly modulate or pulse signals.	(Marti-Figueroa & Ashton, 2017; Zhang et al., 2026)
	Light-controlled clustering or	Potentially single-cell	Millisecond-to-hour timescales;	hESC-derived monolayers	Requires genetic modification and optical hardware; deep-tissue	

Optogenetic activation (e.g., optoWnt)	conformational change activates the signalling pathway in illuminated cells.	resolution in 2D. In 3D organoids, depth depends on the wavelength: standard blue light is limited to ~100–300 μm , while redshifted/far-red light can penetrate up to ~1 mm	arbitrary patterns and duty cycles are programmable.	and embryoid bodies; early-stage organoids; thin or translucent constructs.	patterning in large organoids requires redshifted tools to overcome penetration limits.	(Hellwarth et al., 2021; Repina et al., 2023)
Chemogenetic synthetic receptors	Engineered receptors respond to synthetic ligands absent from the endogenous milieu.	Defined by ligand distribution method (microfluidic, hydrogel, or diffusion); in principle, high.	Tunable through ligand dosing schedules.	Broad, including organoids where genetic modification is feasible.	Off-target pharmacology; need for careful Pharmacokinetics/pharmacodynamics (PK/PD) modelling in 3D tissues.	(Prochazka et al., 2022; Trentesaux et al., 2023)
DNA microbeads and hydrogels	Nanoengineered DNA beads injected into organoids release tethered morphogens upon light or chemical triggers.	Intrinsic localisation within organoid; gradients emanate from the bead on 50–200 μm scales.	High control via photocleavable linkers and bead design; repeated triggering possible.	Retinal organoids; potentially generalizable to other organoid types.	Microinjection labour; potential immunogenicity; regulatory novelty.	(Afting et al., 2024)

Note. Data synthesised from O’Grady et al. (2019), Yaman & Ramanathan (2023), Repina et al. (2023), Trentesaux et al. (2023), and Afting et al. (2024).

When critically comparing these platforms, distinct trade-offs between precision, reproducibility, and scalability become evident. Microfluidic devices offer unmatched spatiotemporal precision and robust gradient maintenance, yet they often suffer from low throughput, complex fabrication, and shear stress-related issues that complicate large-scale manufacturing. Conversely, hydrogel-based systems excel in scalability and 3D organoid compatibility, but they lack the sharp boundary control and rapid reversibility seen in microfluidics. Optogenetics provides unparalleled non-invasive, dynamic control with high resolution, but it is fundamentally limited by the depth of light penetration in dense tissues and requires extensive genetic modification. Finally, DNA microbeads present a highly localised, programmable solution from within the tissue itself, though their reliance on manual microinjection currently restricts reproducibility and high-throughput applications.

4. Organoid Systems Amenable to Gradient Engineering

4.1. Cerebral and Brain Organoids

Brain organoids are ideal platforms for synthetic gradient engineering, as endogenous neural differentiation is strongly dependent on multiple axes of morphogens. Through orthogonal Wnt and Shh gradients, researchers have been able to successfully pattern forebrain, midbrain and hindbrain regions that match human foetal atlases. Moreover, the "fine-tuning" of critical morphogen concentrations can selectively regulate the regional cell fate composition of brain organoids. [Table 3](#) provides a summary of current patterning challenges and opportunities to engineer gradients specific to brain and other organoid models. Yet, these studies also highlight important limitations, such as individual variability and line-to-line differences in morphogen responses, that produce patterning differences.

Moreover, strong and/or global application of high levels of morphogens often obliterates fine-scale organisation, yielding so-called "monoclonal" fates ([Sanchís-Calleja et al., 2026](#); [Scuderi et al., 2025](#)). Finally, gyri, fascicles, and laminar structures are poorly mimicked even in organoids with correctly specified regions, suggesting that by itself, morphogen gradients cannot produce all of the neural cytoarchitecture and that mechanical and activity-dependent processes play an essential role ([Ahmed & Kalangi, 2024](#); [Yao et al., 2024](#)). Thus, gradient engineering in brain organoids provides a robust means to specify regional identities but only a partial means to establish higher-order cytoarchitecture.

4.2. Intestinal Organoids

Intestinal organoids are well-positioned to benefit from engineered gradients because, in vivo, they exhibit distinct crypt and villus domains with opposing underlying Wnt/BMP gradients ([Xiang et al., 2024](#); [Xu et al., 2025](#)). Conventionally, intestinal organoids develop budding crypt-like structures and central lumens; however, they are rarely observed across long-range directions or with villus-like morphologies like those found in vivo ([Huang et al., 2021](#); [Xiang et al., 2024](#)). Theoretically, microfluidic- or hydrogel-based gradients of Wnt agonists, BMPs, and Notch modulators could promote the development of long-range crypt-villus axes, but few studies have been reported (compared to neurones) ([Marti-Figueroa & Ashton, 2017](#); [O'Grady et al., 2019](#); [Xiang et al., 2024](#)).

Recent reviews are right to stress that the adoption of microfluidics, genome editing, and innovative hydrogels is rapidly expanding the utility of intestinal organoids for disease modelling, drug screening and therapies ([Xiang et al., 2024](#); [Xu et al., 2025](#); [Yao et al., 2024](#)).

4.3. Kidney Organoids

Organoids derived from human pluripotent stem cells with nephron-like units comprising podocytes, proximal and distal tubules, and collecting ducts typically have patterning and segmentation along the corticomedullary axis that are scrambled in comparison to a native kidney ([Ahmed & Kalangi, 2024](#); [Huang et al., 2021](#)). Wnt, FGF, and BMP gradients orchestrate segmentation of nephrons in vivo, so engineered gradients could help optimise segmentation and alignment of tubuli in vitro ([Meng et al., 2025](#); [Yao et al., 2024](#)). However, so far, kidney organoid studies have typically modulated these pathways temporally and homogeneously.

Microfluidics and gradient hydrogels can be used to impose, for example, proximal versus distalising cues along the x-axis of a kidney organoid, and synthetic circuits that sense combinations of RA, Wnt and FGF may coordinate segment-specific gene expression (Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). The sparse gradient-engineered studies in kidney organoids can be attributed to technical difficulties, including organoid fragility and 3D structure and the lack of effective readouts of segment identity in 3D. The combination of gradient engineering and better readouts of segment identity, such as spatial transcriptomics and multiplexed imaging, will likely see increased development of gradient-engineered progenitors into nephrons in the future.

4. 4. Cardiac and Vascular Organoids

Heart organoids and engineered heart tissues have made rapid progress, exhibiting chamber-like structures, beating capabilities, and simple vascular networks (Huang et al., 2021; Yao et al., 2024). Wnt and BMP processes play vital roles in early cardiac mesoderm differentiation and later atria versus ventricle differentiation, while VEGF and Notch processes shape blood vessels (Hellwarth et al., 2021; Meng et al., 2025; Yao et al., 2024). Optogenetic tools, such as optoWnt, have been used early in differentiation to induce cardiac mesoderm patches that subsequently form patterned heart muscle (Hellwarth et al., 2021; Repina et al., 2023).

However, strong chamber specification and orientation, like in the four-chambered human heart, have been elusive. This challenge is partly due to the complex interplay between mechanical factors and gradients in heart development; growth and form, lumen (hole) formation, and blood flow interfere with the reading of gradients (Hellwarth et al., 2021; Yao et al., 2024). In this case, engineered gradient reading may have to be complemented with mechanical cues, such as stretch and flow, as well as bioelectricity, to achieve functionally realistic architectures. Vascular organoids that form microvessel networks are a more accessible platform for VEGF gradient engineering, and they have been used to build pre-vascularised tissue grafts (Huang et al., 2021; Yao et al., 2024).

4. 5. Lung and Hepatic Organoids

Lung organoids model proximal–distal patterning, branching morphogenesis, and alveolar differentiation, which are processes known to depend on gradients of FGF, Wnt, BMP, and RA (Meng et al., 2025). However, most lung organoid protocols use uniform factor exposure, resulting in mixtures of proximal and distal cell types without clear spatial segregation (Meng et al., 2025). Synthetic gradients, implemented either through microfluidic chips mimicking airway–alveolar transitions or through hydrogel-embedded growth-factor sources, could help impose more physiological proximal–distal organisation and branching geometries.

Microfluidic liver-on-chip systems have begun to implement oxygen and nutrient gradients, but explicit morphogen gradient programming in hepatic organoids remains rare (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Interestingly, CRISPR-based editing and patient-derived hepatic organoids are already being deployed for disease modelling and drug screening, suggesting that integrating synthetic gradient engineering into these genetically edited, patient-specific models could yield zonation-aware pharmacology platforms (Pulecio et al., 2017; Yao et al., 2024).

Table 3. Organoid Systems and Their Current Patterning Limitations Addressable by Synthetic Gradient Engineering.

Organoid type	Target tissue architecture	Key patterning signals required	Current patterning limitation	Synthetic engineering opportunity	Representative references
Cerebral/brain organoids	Regionalised forebrain, midbrain, and hindbrain with proper cortical layering and DV domains.	Wnt, Shh, BMP, FGF8, RA gradients along AP and DV axes.	Stochastic regionalisation; incomplete laminar structure; poor long-range connectivity.	Orthogonal microfluidic gradients (Duo-MAPS), optogenetic Wnt, and synthetic circuits that sharpen regional boundaries.	(Sanchís-Calleja et al., 2026; Scuderi et al., 2025)
Intestinal organoids	Organised crypt–villus units with aligned axes and appropriate stem cell and differentiated cell zoning.	Wnt/BMP countergradient; Notch modulation; EGF support.	Budding crypt-like structures without consistent villus alignment; batch variability.	Microfluidic or hydrogel-based Wnt/BMP gradients along a single axis; Wnt/BMP-sensitive synthetic gene circuits to reinforce crypt vs villus fates.	(Xiang et al., 2024; Xu et al., 2025)
Kidney organoids	Repeated nephron units with correct segment order (glomerulus, proximal, distal, collecting duct) and cortico-medullary organisation.	Wnt, BMP, FGF, RA gradients; Notch for segmentation.	Disordered segment arrangement; absence of a clear cortico-medullary axis.	Gradient hydrogels delivering proximalizing vs distalizing cues; CRISPR-based reporters and circuits to enforce segment identities.	(Yao et al., 2024; Zhang et al., 2026)
Cardiac organoids	Chambered structures with aligned atrial and ventricular regions and integrated vasculature.	Wnt, BMP, RA, VEGF, Notch gradients; mechanical cues.	Incomplete chamber specification; limited spatial separation of cardiomyocyte subtypes.	Optogenetic Wnt gradients during early mesoderm induction; VEGF gradients to guide vascular network topology.	(Hellwarth et al., 2021; Yao et al., 2024)
Vascular organoids	Hierarchical vascular trees with perfusable lumens and appropriate tip/stalk cell patterning	VEGF, Notch, Angiopoietin gradients.	Isotropic microvessel networks lack hierarchical organisation.	Microfluidic VEGF gradients; Notch-modulating circuits in endothelial cells to stabilise tip/stalk decisions.	(Zhang et al., 2026)
Lung organoids	Proximal–distal airways with branching bronchioles and alveolar regions.	FGF, Wnt, BMP, RA gradients; mechanical stretch.	Mixed proximal and distal cell types without clear spatial segregation.	Organ-on-chip devices imposing FGF/Wnt gradients along the channel; hydrogel scaffolds with patterned morphogen sources.	(Meng et al., 2025)
Liver organoids	Zonation along periportal–pericentral axis with metabolic and signalling gradients; organised bile duct networks.	Wnt, oxygen, nutrient, and hormone gradients; Notch for ductal patterning.	Limited zonation; homogeneous hepatocyte-like populations.	Microfluidic oxygen and Wnt gradients; synthetic circuits reading oxygen and Wnt to enforce zonal gene expression.	(Yao et al., 2024)
Retinal organoids	Stratified retinal layers with correct distribution of photoreceptors, ganglion, and pigment epithelium.	Wnt, Shh, RA gradients; intrinsic Notch signalling.	Incomplete layering; limited pigment epithelium formation.	DNA microbead-based internal Wnt gradients to drive localised pigment epithelium while preserving neural retina.	(Afting et al., 2024)

Note. Data synthesised from Xiang et al. (2024), Yao et al. (2024), Sanchís-Calleja et al. (2025), Afting et al. (2024), and Xu et al. (2025).

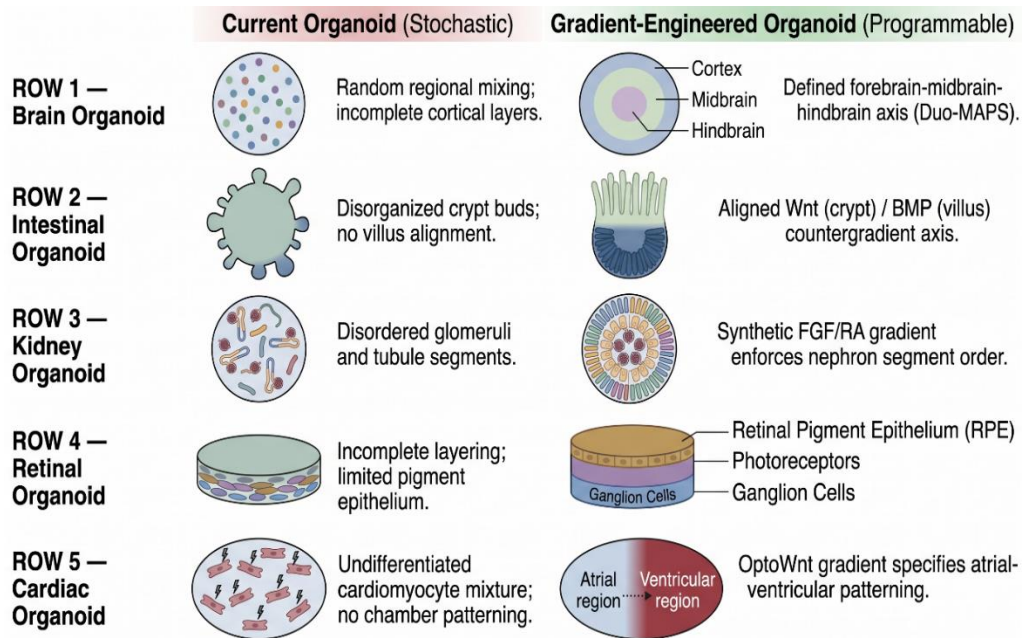


Figure 3. Structural advantages of programmable organoids. A comparison demonstrating how gradient engineering (right) enforces organised cellular patterning and defined spatial axes across various tissue types, overcoming the disorganised, random mixing typical of standard stochastic organoids (left).

5. Molecular Circuits for Gradient Computation in Living Cells

5. 1. Synthetic Gene Circuits Sensing Morphogen Thresholds

Microfluidic liver-on-chip has started to apply oxygen and nutrient gradients, and explicit morphogen gradient programming in hepatic organoids is uncommon (Ahmed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Remarkably, CRISPR-based editing and patient hepatic organoid models are already being implemented to model diseases and screen drugs, implying that integration of systematic gradient engineering into such genetically engineered, patient-specific models could result in zonation-aware pharmacology scaffolds (Pulecio et al., 2017; Yao et al., 2024). To fully maximise the potential of these organoids, scientists can engineer their cells with synthetic circuits that automatically detect and respond to local chemical signals. As an example, artificial gene networks that can respond to changes in local oxygen and Wnt concentrations have the potential to impose strict zonal gene expression, thereby autonomously stabilising the tissue architecture to environmental noise. Table 3 will give a comprehensive description of how these molecular circuits and external gradient tools can ameliorate existing constraints in patterning hepatic and other organoid systems.

Prochazka and others modified circuits in human pluripotent stem cells that commit to readings of combinations of endogenous microRNAs (as cell state proxies) and enable optimisation of output protein levels, including developmental morphogens such as BMP4 (Prochazka et al., 2022). Combined with exogenous BMP addition, these circuits enabled greater control over the composition of the germ layers of the organism, implying a modular approach where morphogen exposure and intrinsic state are combined to inform fate choices (Prochazka et al., 2022). Based upon these designs, programmable circuits may be extended into a secondary morphogen or adhesion molecule only after a cell has undergone a specific extent and duration of an exogenous gradient, which helps to sharpen boundaries within tissues.

5. 2. Bidirectional Feedback Loop Design: Bistable Switches and Oscillations

Feedback is often needed to stabilise fates and form dynamic patterns, like segmentation clocks, through developmental systems (Green & Sharpe, 2015; Wolpert, 1969). Sharp on/off domains can be recapitulated by synthetic bistable switches that consist of mutually repressive transcription factor pairs, amplifying positional information by extending the range of

concentrations in which cells that self-transcribe distinct fates are stable (Dupin et al., 2022; Lam et al., 2022). Experimental and modelling of synthetic multicellular assemblies of drops containing two-node feedback circuits have measured the influence of gradient shape, noise and circuit parameters on positional information, and they found trade-offs between precision and robustness (Dupin et al., 2022).

Gradient phase-locking oscillatory circuits, such as synthetic Notch-based loops or repressilator-like loops, could theoretically be phase-locked to external gradients to form stripe or segment patterns akin to somitogenesis. Although direct testing of such circuits in human organoids is relatively scarce, recent organoid experiments of the effects of segmentation clock-like oscillations in response to relevant morphogen environments have indicated that synthetically amplifying such endogenous oscillators is accessible (Anand et al., 2023; Yaman & Ramanathan, 2023). Importantly, designs with multiple feedback loops need tuning to avoid undesired dynamics like spurious oscillations or multi-stability that are unmatched to the desired patterns.

5. 3. Orthogonal Receptor Engineering

One of the key issues in tissue engineering is how to control particular cell pathways which cannot cause intracellular cross-talk in the sophisticated native signalling context. To overcome it, scientists use orthogonal receptor-expressed proteins that bind only synthetic ligands or non-native epitopes to overlay artificial gradient systems and clean overlaying normal networks (Trentesaux et al., 2023). An example would be the methods described by Trentesaux and colleagues, in which an arbitrary molecule, such as secreted Green Fluorescent Protein (GFP), is turned into a functional morphogen by expressing a chimeric receptor containing a GFP-binding nanobody in conjunction with an intracellular signalling (Trentesaux et al., 2023). With designed controls of receptor affinity, expression levels, downstream effectors, and their combination, scientists are striving to create highly accurate, gradient-responsive behaviours, which are completely independent of natural ligand distributions (Trentesaux et al., 2023).

Such orthogonal systems might be used in organoids to incorporate microfluidic or DNA-bead-based sources of synthetic ligands to realise clean, quantifiable gradients without the confounding influences of endogenous ligand production and consumption. Further, orthogonal morphogens decrease pleiotropic side effects, which is critical for translational safety. A practical problem is how to integrate these receptors without overloading the cellular signalling machinery or putting them into unwanted cross-talk with the scaffold proteins and the subsequent networks.

5. 4. Positional Information CRISPR-Based Epigenetic Recordings

CRISPR-based epigenome editing is not only confined to non-chromatin or chromatin states of static transcriptional repression or activation, but it could also serve to engrave environmental histories in DNA (Shi et al., 2025). Morphogen-responsive promoters can be coupled with CRISPR base editors or dCas9-linked epigenetic enzymes, record cumulative exposure to a gradient as a stable mark, which is subsequently read by sequencing or imaging (Shi et al., 2025). For example, a Shh- or Wnt-responding promoter-driven base-editing guide RNA could encode a positional barcode of historic gradient levels, which, even when the gradient decays, would allow recapitulation of past gradient dynamics.

Although the majority of currently used CRISPR-based epigenetic tools have been used to regulate gene expression in vivo or investigate disease pathophysiology, their use to develop organoid-like structures is conceptually uncomplicated and technically implementable considering existing infrastructures (Pulecio et al., 2017; Shi et al., 2025). Notably, these recording circuits need not per se affect fate but can be implemented as passive sensors, maintaining native developmental dynamics, but with strong readouts to gradient-engineering experiments. This strategy may, in the future, allow the generation of closed-loop applications where the stored positional data proactively influences the future cell fate choices via programmable changes in epigenetics.

5. 5. Juxtacrine and Paracrine Signalling

The synthetic juxtacrine and paracrine signalling circuit consists of cell-cell communication pathways. Lateral inhibition and community effects correlate with gradient reading; tissue patterns are heavily influenced by them (Green & Sharpe, 2015; Wolpert, 1969). Positional information can be enhanced, and boundaries refined by synthetic cell-cell communication circuits driven by engineered juxtacrine signalling, such as synthetic Notch (synNotch) or paracrine circuits. The user-defined transcriptional program can be activated by SynNotch receptors, in which a cell responds when it interacts with a neighbour presenting a particular surface ligand. Gradients or domains of synNotch–ligand complexes can create patterns in which cells change fate based on the local composition of their neighbourhood (Morsut et al., 2016; Trentesaux et al., 2023).

In patterned organoids at gradients, these circuits may help transduce a continuous morphogen field into discrete domains by imposing self-exclusive cell fates that not only depend on their own morphogen exposure but also that of their neighbours. An example is by having cells surpassing a Wnt threshold to express a synNotch ligand that, when contacted, causes adjacent subthreshold cells to switch their fate to a complementary one, sharpening interfaces. Initial experiments in mammalian cell aggregates indicate that synthetic cell-cell communication can induce self-organised patterning and can be translated to human organoids (Morsut et al., 2016; Trentesaux et al., 2023).

6. Bench-to-Bedside Therapeutic and Translational Implications

6. 1. Ready-to-Be Transplanted Organoids and Patterning Fidelity

To be effective in clinical transplantation, organoid-derived grafts need to achieve specific tissue-relevant structures to achieve appropriate host integration, perfusion, and functioning (Ahmed & Kalangi, 2024; Huang et al., 2021; Yao et al., 2024). Providing an alternative design strategy, synthetic gradient engineering is an incredibly potent strategy to pre-pattern these key geometries, including aligned intestinal crypts or layered retinal tissue, potentially overcoming the structural constraints of stochastic self-assembly (Figure 3) (Afting et al., 2024; Xiang et al., 2024). Nevertheless, even with this promise, there is an urgent need to conduct systematic preclinical studies that would satisfactorily compare the long-term engraftment results of the grafts grown by the light of gradient engineering and the traditional growth. An overall summary of these translational applications and barriers to development is outlined in Table 4.

6. 2. Monogenic Disease Modelling and Patient-Derived Organoids

Patient-derived organoids have been positioned at the core of the organoid disease modelling of monogenic diseases, such as cystic fibrosis, liver metabolic diseases, and inherited kidney diseases (Alnasser, 2025; Xiang et al., 2024; Yao et al., 2024). In these conditions, like cystic fibrosis, liver metabolic diseases, and inherited kidney diseases, structural defects typically occur not only at the cellular scale but also within the broader tissue architecture, leading to altered crypt-villus ratios, disorganised bile duct networks, or maladaptively formed nephron segments. (Ahmed & Kalangi, 2024; Huang et al., 2021; Yao et al., 2024). Synthetic morphogen gradients provide a means of standardising space, on which such mutations are examined, avoiding confounding variability in individuals caused by disorganised patterning.

Also, gradient engineering could enable patterning correction strategies in which patient-derived organoids with a repaired genotype, using CRISPR-Cas9 editing, i.e., exposed to standardised morphogen programmes, would determine whether restored signalling is converted into normal tissue architecture (Pulecio et al., 2017; Yao et al., 2024). This method, in particular, is useful in investigating diseases that are the result of mutations that impair the capacity of a cell to respond to morphogens. By putting the mutant cells on board with the genetically corrected cells, researchers are in a position to expose the mutant cells to the same gradients as the genetically corrected cells. This direct and side-by-side comparison will enable the scientists to chart out precisely the development of tissues in relation to the presence of a particular genetic mutation.

6. 3. Pharmacodynamic Testbeds and Drug Screening

Organoids are also available in an increasing variety of drug screening and toxicity tests, but they are considered uniform tissues (Alnasser, 2025; Huang et al., 2021; Wang et al., 2025; Yao et al., 2024). Nonetheless, in vivo distribution and response of drugs to various tissue regions delimited by morphogen and metabolic gradients, such as periportal versus pericentral hepatocytes or crypt stem cells versus villus enterocytes, are challenging (Huang et al., 2021; Yao et al., 2024). It can also be more realistic and spatially resolved, so gradient-patterned organoids can give a drug response.

Microfluidic organoid-on-chip systems can subject different parts of an organoid or organoid array to a discussable amount of various drugs, but internal morphogen gradients mean that they can read out region-specific toxicity and efficacy (Ahammed & Kalangi, 2024; Alnasser, 2025). One practical application is the use of intestinal organoids patterned by synthetic Wnt/BMP gradients to model gastrointestinal toxicity. Researchers propose that this system can elucidate the preferential damage chemotherapeutics inflict on crypt stem cells compared to villus cells, thereby guiding strategies to mitigate mucositis (Xiang et al., 2024; Xu et al., 2025). On the same note, liver organoids reconstituted to be zonated are suggested to become a way to narrow down the predictions of drug-induced liver injury by probing the zone-specific susceptibility (Yao et al., 2024).

6. 4. Organ-on-Chip Integration

Microfluidics, synthetic biology, and tissue engineering: an obvious overlap of gradient-engineered organoids with organ-on-chip platforms is a logical progression (Ahammed & Kalangi, 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019). These systems enable sustained perfusion, mechanical feedback (e.g., stretch or fluid shear), and intricate biochemical gradients and allow providing high-content imaging capabilities and sampling (Ahammed & Kalangi, 2024; Alnasser, 2025; Wang et al., 2025). Organoid-on-chip devices have already enhanced oxygenation, maturation, and vascularisation in various models, and more frequently include morphogen gradients in diverse models to recapitulate developmental microenvironments (Ahammed & Kalangi, 2024; Yao et al., 2024).

In the context of regenerative medicine, organoid-on-chip platforms may play the role of pre-conditioning bioreactors in which grafts are conditioned via gradient programmes that are defined to shape architecture and behaviour before transplant. Applying multiplexed chips with organoid arrays of gradient patterns of individual patients to precision medicine pipelines that capture realistic tissue heterogeneity would support drug discovery. The difficulty is to harmonise during the connectivity between chips and organoids, control the batch-to-batch variation, and meet the regulatory demands of the device-tissue assemblies.

6. 5. Regulatory, Manufacturing, and Clinical Limitations

From a regulatory and manufacturing perspective, scaling up gradient-engineered organoids introduces distinct challenges. Although programmable gene circuits and CRISPR-based positional recordings present revolutionary theoretical possibilities for custom tissue design, their current application remains strictly experimental. Fully programmed tissue structures have yet to be realised in human clinical trials, and researchers must carefully distinguish between these conceptually supported models and experimentally validated applications.

Translating these technologies into the clinic will require overcoming significant manufacturing bottlenecks, particularly regarding scalability and reproducibility across massive batches. Furthermore, long-term clinical application is complicated by biological risks like insertional mutagenesis, off-target genetic effects, and immunogenicity against engineered or foreign proteins. Ethical considerations also remain a significant hurdle, particularly concerning the development of advanced, regionally specified brain organoids. Finally, DNA-based materials and microfluidic chips require rigorous biocompatibility and sterility validations before transitioning from the lab bench to human patients. Because of these limitations, practical clinical pathways will likely adopt a progressive approach, beginning with *ex vivo* diagnostics and drug screening, before eventually advancing to direct patient transplantation.

Table 4. Translational Applications of Gradient-Patterned Organoids Across Disease and Therapeutic Contexts.

Application area	Disease/condition	Organoid system	Gradient engineering approach	Stage of development	Major translational barrier	Representative references
Regenerative transplantation	Intestinal failure, short bowel syndrome	Intestinal organoids	Wnt/BMP countergradients to align crypt–villus architecture before engraftment.	Preclinical concepts; limited <i>in vivo</i> engraftment studies.	Achieving scale, vascularisation, and functional integration with host mucosa.	(Marti-Figueroa & Ashton, 2017; Xiang et al., 2024)
Regenerative transplantation	Retinal degeneration	Retinal organoids	DNA microbead–mediated internal Wnt gradients to generate layered retina and pigment epithelium.	Preclinical in animal models; <i>in vitro</i> optimisation ongoing.	Long-term survival, synaptic integration, and immune compatibility.	(Afting et al., 2024)
Disease modelling and drug testing	Cystic fibrosis, Inflammatory Bowel Disease (IBD), Colorectal Cancer (CRC)	Intestinal and colonic organoids	Microfluidic and hydrogel gradients of inflammatory cytokines and drugs along crypt–villus axes.	Widely used organoids; gradient engineering is emerging.	Standardisation and high-throughput compatibility; patient variability.	(Xiang et al., 2024; Xu et al., 2025)
Metabolic and liver disease	Non-Alcoholic Fatty Liver Disease (NAFLD), Non-Alcoholic Steatohepatitis (NASH), hepatotoxicity	Liver organoids and liver-on-chip	Oxygen/nutrient and Wnt gradients to model zonation; drug concentration gradients.	Organ-on-chip devices in advanced preclinical use.	Complex culture systems; regulatory acceptance of microfluidic platforms.	(Yao et al., 2024)
Neurodevelopmental disorders	Microcephaly, autism spectrum, epilepsy	Brain organoids	Orthogonal Wnt/Shh gradients to pattern regions; optogenetic circuits to model activity-dependent development.	Brain organoids are already used extensively in gradient systems in early-stage studies.	Ethical issues, model variability, and limited maturation.	(Sanchís-Calleja et al., 2026; Scuderi et al., 2025)
Cardiovascular disease	Cardiomyopathies, arrhythmias	Cardiac organoids and engineered heart tissues	Wnt/BMP gradients during mesoderm induction; VEGF gradients for vascularisation; optogenetic patterning of conduction tissue.	Preclinical models for drug testing and disease modelling.	Reproducing mature conduction systems and chamber architecture.	(Hellwarth et al., 2021; Yao et al., 2024)
Precision oncology	Tumour heterogeneity and microenvironment	Tumour organoids	Gradients of growth factors, oxygen, and	Patient-derived tumour organoids	Modelling immune and stromal	

Gene and cell therapy development	Vector targeting and off-target toxicity	and co-culture systems	drugs to mimic tumour cores vs margins.	in early clinical decision-making.	components; intratumoral heterogeneity.	(Alnasser, 2025)
		Multi-organoid arrays	Controlled gradients of viral vectors or genome editors across organoid systems on chips.	Conceptual and early experimental work.	Biosafety of gene editing; integration with manufacturing workflows.	(Pulecio et al., 2017; Shi et al., 2025)

Note. Data synthesised from Xiang et al. (2024), Yao et al. (2024), Afting et al. (2024), Marti-Figueroa & Ashton (2017), and Ahammed & Kalangi (2024).

7. Future Perspectives: The Era of Programmable Organoids

Looking forward, the transition from stochastic self-organisation to fully programmable tissue engineering represents a paradigm shift in regenerative medicine. We envision a future where patient-derived stem cells are combined with "smart" microfluidic scaffolds and synthetic genetic circuits that automatically guide their own development. In this framework, transient external gradients will reliably initiate early symmetry breaking, while internal, CRISPR-recorded genetic switches will permanently lock in mature tissue architectures. Ultimately, integrating these programmable organoids into scalable, closed-loop bioreactors will bridge the gap between basic developmental biology and the reliable mass manufacturing of personalised, transplantable organs.

8. Critical Synthesis and Analysis

8. 1. Emerging Patterns of Convergence

There are a few trends that emerge from papers that use the various platforms and organoid models described above. First, gradient engineering of any sort (e.g., microfluidics, hydrogels, optogenetics or DNA microbeads) always increases control of fate speciation in organoids versus simple feeding with medium (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023; Trentesaux et al., 2023). This impact is variable depending on the tissue type and culture conditions, but the trend is very consistent. Second, platforms that engineer gradients and also provide at least some basic synthetic circuitry or stateful reporters mediate more intuitive and reproducible outcomes because they directly couple environmental profiles to genetic decision-making and readouts (Dupin et al., 2022; Prochazka et al., 2022; Shi et al., 2025; Trentesaux et al., 2023). Third, combined strategies that employ engineered gradients to bias early symmetry breaking and leave the hard work of development to intrinsic programmes seem to be more effective than attempts to impose pre-patterning on pre-existing developmental programmes in a completely deterministic fashion (Anand et al., 2023; Repina et al., 2023; Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023).

In all, these three emerging trends suggest that the field is coming to a consensus that synthetic gradients do not replace development; rather, they steer it. They constrain self-organisation and leave fine-scale architecture to the cells themselves. Engineered gradients are shifting from controlling development to guiding it.

8. 2. Paradoxes and Controversies

As the field comes together on these underlying principles, there are considerable debates and challenges. A common question is the level of engineered control desirable for self-organisation. Microfluidic experiments demonstrate that there are strong, persistent exogenous gradients that can override endogenous patterning to produce tissues with natural-looking but somewhat forced domain boundaries or unnatural lineage distribution (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Scuderi et al., 2025). Others report that, despite well-calibrated gradients, organoids show variable results, with evidence of stochastic fluctuations and line variability, suggesting that this variation may be unavoidable or even beneficial (Anand et al., 2023; Sanchís-Calleja et al., 2026; Yaman & Ramanathan, 2023).

There is also no consensus about the complexity of gradient programmes. Some argue that rather simple, monotonic gradients of one or two morphogens are sufficient, as endogenous regulatory networks will be capable of filling in the gaps. Others envision more and more complex gradients with multiple morphogens and time-dependent profiles, hoping to mimic the dynamics of the embryo (O'Grady et al., 2019; Sanchís-Calleja et al., 2026; Trentesaux et al., 2023). Synthetic biology introduces yet another dimension, with some designs opting for simple circuits that minimally interfere with endogenous networks, whereas others aim to build very sophisticated engineered cell lines with extensive orthogonal recording and signalling capacities (Prochazka et al., 2022; Shi et al., 2025; Trentesaux et al., 2023). The right balance between simplicity, robustness and faithfulness is unclear.

8. 3. Technological Challenges: Reproducibility, Scalability, and Vascularisation

Technically, many gradient-engineered organoid studies are proof-of-concept studies involving small numbers of organoids, implementing gradient techniques that are technically difficult to replicate elsewhere, such as custom-designed setups (Afting et al., 2024; Marti-Figueroa & Ashton, 2017; O'Grady et al., 2019; Repina et al., 2023). Sources of variability include changes in the stem cell differentiation and culture matrix (for instance, lots of Matrigel) (Ahammed & Kalangi, 2024; Huang et al., 2021; Wang et al., 2025). Very few studies evaluate the influence on variability of gradient parameters like slope, amplitude, and time, or include robust statistical designs and quality of pattern evaluations by blinded third parties.

Challenges in reproducing these systems aside, a key challenge is how to translate these technologies into manufacturing at scale. Microfluidic chips and microinjection-based DNA beads are amazing for exploring biology but are hard to scale up to screen or manufacture hundreds or thousands of organoids (Afting et al., 2024; O'Grady et al., 2019; Trentesaux et al., 2023). Hydrogels containing channels or beads can be scaled up, but they lose resolution (Marti-Figueroa & Ashton, 2017; Zhang et al., 2026). Another common problem is a lack of vascularisation; without perfusable networks, large organoids will develop necrotic cores and unnatural oxygen and nutrient gradients that hinder patterning by morphogen gradients (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). Even gradient-engineered vascular organoids have yet to be routinely integrated into other organoids to alleviate this issue at scale.

8. 4. Closing the Gap: Immune Stromal and Gradient Microenvironments

Although the first series of organoid models, created by Sato and co-workers in 2009, has laid the foundation for organoid models made purely of epithelium, the overwhelming majority of studies in the past 15 years continue in this vein, still primarily focused on parenchymal tissues (Sato et al., 2009; Yao et al., 2024). As a result, there remains a paucity of immune and stromal cell types critical for the correct functioning of mature tissues. The inclusion of these cells remains a major challenge because cell lineages frequently require different (and, at times, competing) morphogen conditions to survive and/or self-organise (Green & Sharpe, 2015; Huang et al., 2021). However, in vivo, fibroblasts, endothelial cells, pericytes, and immune cells respond to and modify the diffusion and transport of morphogens by releasing ligands, antagonists, and extracellular matrix (ECM) components (Ahammed & Kalangi, 2024; Alnasser, 2025; Meng et al., 2025). In the absence of these factors in most gradient-engineered organoids, it is likely that effective gradients are modified and may blunt the maturation and stability of the patterned tissue.

New types of co-culture organoids with integrated immune and stromal cells, and even microbiota, have started to shed light on how these interactions influence organoid function, but gradient engineering in these more complex organoid systems is just beginning (Alnasser, 2025; Xiang et al., 2024; Xu et al., 2025). An important avenue for future research is to engineer gradients of both traditional morphogens and chemokines, cytokines, and ECM gradients to recapitulate immune-stromal-parenchymal communication. This would be especially important for tissue regeneration strategies, where grafts will have to respond to host gradients of immune and stromal cues.

8. 5. Can Existing Gradient Tools Pattern Tissues?

The question arises whether synthetic gradient tools have now matured to a level of refinement at which they can be used for tissue patterning, much as they occur in development, or whether they are merely "toy" systems. These tools appear to have varying degrees of success. For example, systems like Duo-MAPS and DNA microbeads are able to provide multi-axis and internal gradients, yielding readily identifiable, patterned tissues (Afting et al., 2024; Scuderi et al., 2025). Also, optogenetic and chemogenetic tools show that selective activation for subsets of cells is sufficient to achieve global self-organisation and boundary formation (Repina et al., 2023; Trentesaux et al., 2023).

By contrast, very few, if any, studies present organoids with global shape, multilayered architecture and functions, such as electrochemical pathways, peristaltic waves, or bile transport, that match in vivo organs as a result of engineered gradients. They typically revolve around regional differentiation and domain formation, rather than whole organ morphogenesis (Ahammed & Kalangi, 2024; Wang et al., 2025; Yao et al., 2024). We must find out whether improvements to our existing platforms, better

microfluidics, more versatile hydrogels, and more reliable optogenetics are enough or whether new ideas, such as integrated closed-loop artificial intelligence-driven cultivation strategies or co-designed mechanical and morphogen fields, will be needed to reach this milestone.

9. Implications

9. 1. Research Implications

There are several evident research challenges arising from this marriage of developmental cues, microfluidic devices and synthetic biology. First, there is a need for systematic gradient design experiments, in which characteristics such as gradient amplitude, slope, and temporal features are systematically adjusted and tested in high-throughput conditions. Ideally, these experiments will involve automated fluorescence imaging and single-cell transcriptomics to reveal how different combinations of multiple morphogens lead to different cell fates (Huang et al., 2021; Sanchís-Calleja et al., 2026; Shi et al., 2025). This will help us progress from anecdotal experimentation toward a quantitative understanding of how gradient features affect pattern formation. Second, when combined with synthetic reporter/recording gene circuits, these experiments will allow novel measurements of how cells sample, integrate and interpret gradients over time to identify information bottlenecks (Dupin et al., 2022; Prochazka et al., 2022; Shi et al., 2025).

Third, certain organoid systems deserve particular attention because of their specific developmental challenges and clinical promise. Best among these are brain organoids, for which the pairing of orthogonal gradients with CRISPR-based molecular time stamping promises a revolutionary approach to investigating neural organization. By leveraging these synthetic circuit tools, we can start to dissect how position is not only defined but also recorded and fine-tuned over the duration of human neurogenesis, which takes place over many weeks (Sanchís-Calleja et al., 2026; Scuderi et al., 2025). This process facilitates the "timestamping" of signalling events and bridges the gap by mapping early exposure to the morphogen gradient as it orchestrates the late-time organisation of cortical layers and long-distance connectivity patterns, which have been intractable to self-organising models to date (Shi et al., 2025; Yao et al., 2024). In addition, focusing on systems that recapitulate cross-lineage interactions, such as neurovascular and liver-immune organoids, will be key to unravelling how the differential effects of patterned signalling control the co-organisation of different cell types in functional tissue units. Intestinal and liver organoids, owing to their clinical relevance, should be natural targets for experiments that link gradient-driven architecture to clinically relevant functions such as barrier function, cholate metabolism, and pharmacokinetics (Alnasser, 2025; Huang et al., 2021; Xiang et al., 2024; Xu et al., 2025; Yao et al., 2024). The kidney and lung organoid communities present formidable challenges with high rewards, where successful improvements in patterning will enable valuable disease models.

Lastly, field standards for reporting gradient patterning studies would be imperative, such as descriptions of device architecture, morphogen dynamics, strategies for gradient measurement, and cell line-specific responses (Huang et al., 2021; Martí-Figueroa & Ashton, 2017; O'Grady et al., 2019). Such reporting will allow for better inter-lab comparisons and the development of a knowledge base.

9. 2. Clinical and Translational Implications

From a clinical perspective, synthetic morphogen-gradient engineering is part of the emerging trend towards programmable, precision regenerative medicine. From a regulatory and translational standpoint, a critical consideration is the duration for which synthetic control must be maintained within the graft. While the long-term maintenance of integrated synthetic networks, such as stably expressed optogenetic systems or synthetic receptors, offers prolonged spatial control, it also introduces substantial clinical risks, including insertional mutagenesis, off-target genetic effects, and long-term immunogenicity against foreign proteins. Consequently, transient "hit-and-run" gradient engineering presents a much more viable path toward Good Manufacturing Practice (GMP) compliance. Biologically, transient application utilising mRNA-based circuits or non-integrating tools during the early differentiation phase is likely sufficient to establish stable tissue architecture. Similar to natural embryogenesis, where morphogen gradients frequently act as temporary cues that trigger symmetry breaking, early transient

engineering can establish foundational spatial coordinates. Once cells cross specific morphogen thresholds, endogenous gene regulatory networks and epigenetic memory "lock in" these committed fates. This allows the foundational architecture, such as a neural axis or a crypt-villus boundary, to be set early, enabling the pre-patterned organoids to undergo subsequent maturation in standard, device-free bioreactors prior to transplantation.

Concerning the organ shortage, gradient-optimised organoids may not be the next organ transplants, but they may be viable candidates for patch/substantial grafts, intestinal sleeves for improved digestion, or liver lobules or patches for metabolic backup, or retina patches for local vision (Alnasser, 2025; Huang et al., 2021; Yao et al., 2024). Realistically, gradient-engineered, clinically transferable grafts for such applications are unlikely to be available for at least another decade of trial-and-error experimentation and safety testing, due to the challenges of integrating the synthetic gradient, gene circuits and host system.

For precision medicine, patient-specific patterning correction is an intriguing idea. We can envisage a process in which patients' organoids are standardised using defined gradient programmes to produce standardised tissue structures, which are subsequently used to screen gene therapies or drug treatments under controlled microenvironmental conditions (Alnasser, 2025; Xiang et al., 2024; Xu et al., 2025). If coupled with CRISPR-based historic recording, this approach can capture long-term reactions and new lineages, supplying vital information to plan patient-specific therapies (Pulecio et al., 2017; Shi et al., 2025).

9. 3. Theoretical and Conceptual Implications

From a theoretical point of view, the engineering of morphogen gradients in organoids has an impact on theories of positional information. Synthetic systems allow independent control of gradient noise, geometry and circuit topology, which are not typically accessible in embryos. This provides fresh insights to test theoretical models that quantify the capacity of a gradient to transmit positional information and the extent of this information used by cells (Dupin et al., 2022; Green & Sharpe, 2015; Turing, 1952; Wolpert, 1969). Preliminary studies with synthetic gradients hint that developmental systems might be closer to the information-theoretic limits than once thought but also give rise to redundancy and feedback enabling robust patterning in the face of considerable noise (Dupin et al., 2022; Lam et al., 2022).

Similarly, the new hybrid model, in which exogenous engineering provides boundary conditions and endogenous development provides details, suggests a more sophisticated model of control in biology. Instead of engineering vs self-organisation, organoids seem to be in an intermediate regime where simple, timely interventions can tip the scales towards desired attractors without predetermining details. A deeper understanding of this balance may help us understand the robustness and evolvability of the natural development process and how far such a process can be pushed in synthetic systems.

10. Limitations of this Review

This review is a narrative one, which explores human organoid systems and synthetic morphogen gradient engineering, rather than including invertebrate and plant systems, despite the impressive capabilities of morphogen gradient engineering in these systems. This means that conceptual and technological advances from non-human systems are only included indirectly in this synthesis. Our survey of the literature highlights peer-reviewed publications to late 2025. Although this field is rich with preprints and conference abstracts, which were not extensively surveyed, it may provide further insights.

The perspective offered here is a specific approach to integrating the literature; we focused on studies that explicitly link gradient engineering to organoid patterning rather than tracing a comprehensive coverage of all synthetic biology literature. Variability in technology platforms, cell types, and analysis methods across studies often precludes direct comparisons, as differences may arise primarily from technical aspects and not biological relevance. Lastly, the translational outlook is based on proof-of-concept, early-stage experiments; regulatory, manufacturing and clinical challenges with these new technologies may be greater than expected.

11. Conclusion

From mathematical descriptions of embryonic development to the control of variables for engineering human tissue in the laboratory, morphogen gradients are poised to move from descriptive to predictive biology. Combining chemical and mechanical

gradient generators with biomaterial and microfluidic technologies, synthetic gene circuits and orthogonal receptors can now be used to allow cells to sense, process and store positional information with a new level of control. The key promise of this technology is to enable organoids to evolve from probabilistic self-organising microstructures to deterministic building blocks in regenerative medicine. Recent findings indicate that, while external gradients enhance regional fate specification, the most robust structures are based on hybrid approaches that integrate artificially engineered cues with self-organised information.

To successfully transition these hybrid models into multi-lineage co-cultures, researchers must address the complex dynamic where native immune and stromal cells act as unpredictable sources and sinks for morphogens, potentially destabilising intended spatial patterns. One viable strategy to preserve patterning fidelity is spatiotemporal staging, utilising transient "hit-and-run" gradients to establish the foundational parenchymal architecture before introducing the immune and stromal compartments, thereby decoupling initial symmetry-breaking from inflammatory cross-talk. Alternatively, orthogonal receptor engineering can bypass this noise entirely. By delivering synthetic ligands that only the engineered parenchymal cells can recognise, the gradient remains perfectly insulated from stromal consumption or interference. For models that must rely on native morphogens, the deployment of dual-axis microfluidic systems, where one axis delivers developmental cues and a secondary axis provides chemokine gradients for immune cell homing, or "smart" DNA-based scaffolds capable of dynamically adapting to cellular consumption will be essential for maintaining precise morphogen thresholds in a complex microenvironment.

Developing this potential over the next decade will demand an integrated design approach to optimise biochemical gradients, mechanics, and blood supplies. In the long run, establishing these guidelines for gradient engineering will not only unleash targeted therapies, such as zonated liver lobules or layered retinal tissues, but also provide the underlying principles for building elaborate human tissues on demand.

Funding

The authors were not funded by anybody to do this work.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No data was used for the research described in the article.

Acknowledgments

Generative AI and AI-Assisted Technologies in Writing. In coming up with this manuscript, the authors used BioRender for figures and Jenni AI to help them with literature discovery and refinement of the English language. After using these tools, the authors thoroughly revised, checked, and edited all material, after which they took full ownership of the accuracy, integrity, and originality of the final publication.

Contributions of the authors

The ideas of the study were drafted, and the study design was developed by E.R.T. and K.K.; the literature review and data curation were conducted by E.R.T., E.S.B. and K.K.; the manuscript was prepared by S.F.N., J.R. and S.G.; the figures were prepared by K.K. and E.S.B.; the manuscript was revised by S.B. and E.R.T.

References

- Afting, C., Walther, T., Drozdowski, O. M., Schlagheck, C., Schwarz, U. S., Wittbrodt, J., & Göpfrich, K. (2024a). DNA microbeads for spatio-temporally controlled morphogen release within organoids. *Nature Nanotechnology*, 19(12), 1849–1857. <https://doi.org/10.1038/s41565-024-01779-y>
- Afting, C., Walther, T., Drozdowski, O. M., Schlagheck, C., Schwarz, U. S., Wittbrodt, J., & Göpfrich, K. (2024b). DNA microbeads for spatio-temporally controlled morphogen release within organoids. *Nature Nanotechnology*, 19(12), 1849–1857. <https://doi.org/10.1038/s41565-024-01779-y>
- Ahammed, B., & Kalangi, S. K. (2024). A Decade of Organoid Research: Progress and Challenges in the Field of Organoid Technology. *ACS Omega*, 9(28), 30087–30096. <https://doi.org/10.1021/acsomega.4c03683>
- Alnasser, S. M. (2025). From gut to liver: Organoids as platforms for next-generation toxicology assessment vehicles for xenobiotics. *Stem Cell Research & Therapy*, 16(1), 150. <https://doi.org/10.1186/s13287-025-04264-y>
- Anand, G. M., Megale, H. C., Murphy, S. H., Weis, T., Lin, Z., He, Y., Wang, X., Liu, J., & Ramanathan, S. (2023). Controlling organoid symmetry breaking uncovers an excitable system underlying human axial elongation. *Cell*, 186(3), 497–512.e23. <https://doi.org/10.1016/j.cell.2022.12.043>
- Barkai, N., & Shilo, B.-Z. (2009). Robust Generation and Decoding of Morphogen Gradients. *Cold Spring Harbor Perspectives in Biology*, 1(5), a001990–a001990. <https://doi.org/10.1101/cshperspect.a001990>
- Dupin, A., Aufinger, L., Styazhkin, I., Rothfischer, F., Kaufmann, B. K., Schwarz, S., Galensowske, N., Clausen-Schaumann, H., & Simmel, F. C. (2022). Synthetic cell-based materials extract positional information from morphogen gradients. *Science Advances*, 8(14), eabl9228. <https://doi.org/10.1126/sciadv.abl9228>
- Gierer, A., & Meinhardt, H. (1972). A theory of biological pattern formation. *Kybernetik*, 12(1), 30–39. <https://doi.org/10.1007/BF00289234>
- Green, J. B. A., & Sharpe, J. (2015). Positional information and reaction-diffusion: Two big ideas in developmental biology combine. *Development*, 142(7), 1203–1211. <https://doi.org/10.1242/dev.114991>
- Hellwarth, P. B., Chang, Y., Das, A., Liang, P., Lian, X., Repina, N. A., & Bao, X. (2021). Optogenetic-mediated cardiovascular differentiation and patterning of human pluripotent stem cells. *Advanced Genetics*, 2(3), e202100011. <https://doi.org/10.1002/ggn2.202100011>
- Huang, Y., Huang, Z., Tang, Z., Chen, Y., Huang, M., Liu, H., Huang, W., Ye, Q., & Jia, B. (2021). Research Progress, Challenges, and Breakthroughs of Organoids as Disease Models. *Frontiers in Cell and Developmental Biology*, 9, 740574. <https://doi.org/10.3389/fcell.2021.740574>
- Lam, C., Saluja, S., Courcoubetis, G., Yu, D., Chung, C., Courte, J., & Morsut, L. (2022). Parameterized Computational Framework for the Description and Design of Genetic Circuits of Morphogenesis Based on Contact-Dependent Signaling and Changes in Cell–Cell Adhesion. *ACS Synthetic Biology*, 11(4), 1417–1439. <https://doi.org/10.1021/acssynbio.0c00369>
- Marti-Figueroa, C. R., & Ashton, R. S. (2017). The case for applying tissue engineering methodologies to instruct human organoid morphogenesis. *Acta Biomaterialia*, 54, 35–44. <https://doi.org/10.1016/j.actbio.2017.03.023>
- Meng, G., Yin, W., Hong, Y., & Oliviero, S. (2025). Emerging roles of epigenetic regulators during lung development. *Cell Death & Disease*, 16(1), 567. <https://doi.org/10.1038/s41419-025-07823-6>
- Morsut, L., Roybal, K. T., Xiong, X., Gordley, R. M., Coyle, S. M., Thomson, M., & Lim, W. A. (2016). Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *Cell*, 164(4), 780–791. <https://doi.org/10.1016/j.cell.2016.01.012>

- O'Grady, B., Balikov, D. A., Wang, J. X., Neal, E. K., Ou, Y.-C., Bardhan, R., Lippmann, E. S., & Bellan, L. M. (2019). Spatiotemporal control and modeling of morphogen delivery to induce gradient patterning of stem cell differentiation using fluidic channels. *Biomaterials Science*, 7(4), 1358–1371. <https://doi.org/10.1039/C8BM01199K>
- Prochazka, L., Michaels, Y. S., Lau, C., Jones, R. D., Siu, M., Yin, T., Wu, D., Jang, E., Vázquez-Cantú, M., Gilbert, P. M., Kaul, H., Benenson, Y., & Zandstra, P. W. (2022). Synthetic gene circuits for cell state detection and protein tuning in human pluripotent stem cells. *Molecular Systems Biology*, 18(11), MSB202110886. <https://doi.org/10.15252/msb.202110886>
- Pulecio, J., Verma, N., Mejía-Ramírez, E., Huangfu, D., & Raya, A. (2017). CRISPR/Cas9-Based Engineering of the Epigenome. *Cell Stem Cell*, 21(4), 431–447. <https://doi.org/10.1016/j.stem.2017.09.006>
- Repina, N. A., Johnson, H. J., Bao, X., Zimmermann, J. A., Joy, D. A., Bi, S. Z., Kane, R. S., & Schaffer, D. V. (2023). Optogenetic control of Wnt signaling models cell-intrinsic embryonic patterning using 2D human pluripotent stem cell culture. *Development*, 150(14), dev201386. <https://doi.org/10.1242/dev.201386>
- Sanchís-Calleja, F., Azbukina, N., Jain, A., He, Z., Okamoto, R., Rusimbi, C., Rifés, P., Rathore, G. S., Santel, M., Janssens, J., Seimiya, M., Eisinger, B., Fleck, J. S., Kirkeby, A., Camp, J. G., & Treutlein, B. (2026). Systematic scRNA-seq screens profile neural organoid response to morphogens. *Nature Methods*, 23(2), 465–478. <https://doi.org/10.1038/s41592-025-02927-5>
- Sato, T., Vries, R. G., Snippert, H. J., Van De Wetering, M., Barker, N., Stange, D. E., Van Es, J. H., Abo, A., Kujala, P., Peters, P. J., & Clevers, H. (2009). Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature*, 459(7244), 262–265. <https://doi.org/10.1038/nature07935>
- Schaerli, Y., Munteanu, A., Gili, M., Cotterell, J., Sharpe, J., & Isalan, M. (2014). A unified design space of synthetic stripe-forming networks. *Nature Communications*, 5(1), 4905. <https://doi.org/10.1038/ncomms5905>
- Scuderi, S., Kang, T.-Y., Jourdon, A., Nelson, A., Yang, L., Wu, F., Anderson, G. M., Mariani, J., Tomasini, L., Sarangi, V., Abyzov, A., Levchenko, A., & Vaccarino, F. M. (2025). Specification of human brain regions with orthogonal gradients of WNT and SHH in organoids reveals patterning variations across cell lines. *Cell Stem Cell*, 32(6), 970-989.e11. <https://doi.org/10.1016/j.stem.2025.04.006>
- Shi, L., Li, S., Zhu, R., Lu, C., Xu, X., Li, C., Huang, X., Zhao, X., Mao, F., & Li, K. (2025). CRISPRepi: A multi-omic atlas for CRISPR-based epigenome editing. *Nucleic Acids Research*, 53(D1), D901–D913. <https://doi.org/10.1093/nar/gkae1039>
- Trentesaux, C., Yamada, T., Klein, O. D., & Lim, W. A. (2023). Harnessing synthetic biology to engineer organoids and tissues. *Cell Stem Cell*, 30(1), 10–19. <https://doi.org/10.1016/j.stem.2022.12.013>
- Turing, A. M. (1952). The chemical basis of morphogenesis. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 237(641), 37–72. <https://doi.org/10.1098/rstb.1952.0012>
- Wang, J., Xia, Z., & Su, J. (2025). Organoid research breakthroughs in 2024: A review. *Organoid Research*, 1(2), 025040005. <https://doi.org/10.36922/OR025040005>
- Wolpert, L. (1969). Positional information and the spatial pattern of cellular differentiation. *Journal of Theoretical Biology*, 25(1), 1–47. [https://doi.org/10.1016/S0022-5193\(69\)80016-0](https://doi.org/10.1016/S0022-5193(69)80016-0)
- Xiang, T., Wang, J., & Li, H. (2024). Current applications of intestinal organoids: A review. *Stem Cell Research & Therapy*, 15(1), 155. <https://doi.org/10.1186/s13287-024-03768-3>
- Xu, X., Zhang, Y., Huang, G., Perekatt, A., Wang, Y., & Chen, L. (2025). Advances and applications of gut organoids: Modeling intestinal diseases and therapeutic development. *Life Medicine*, 4(2), lnaf012. <https://doi.org/10.1093/lifemedi/lnaf012>
- Yaman, Y. I., & Ramanathan, S. (2023). Controlling human organoid symmetry breaking reveals signaling gradients drive segmentation clock waves. *Cell*, 186(3), 513-527.e19. <https://doi.org/10.1016/j.cell.2022.12.042>

- Yao, Q., Cheng, S., Pan, Q., Yu, J., Cao, G., Li, L., & Cao, H. (2024). Organoids: Development and applications in disease models, drug discovery, precision medicine, and regenerative medicine. *MedComm*, 5(10), e735. <https://doi.org/10.1002/mco2.735>
- Zhang, C., Shen, Y., Huang, M., Wang, G., Miao, Q., Shi, H., Gao, R., Wang, K., & Luo, M. (2026). Dynamic hydrogel mechanics in organoid engineering: From matrix design to translational paradigms. *Bioactive Materials*, 55, 144–170. <https://doi.org/10.1016/j.bioactmat.2025.09.021>



Radiologic Evaluation of the Upper Airway Before Anaesthesia

Hanan A. Al-Haidari ^{1*} , Ali Sayer Abbas ¹, Kawther H. Al-khafaji ² , Mustafa Muhammad Ubaid ¹,
Ghofran Majid Waheed ¹, and Alya Riad Abd ¹



¹ Islamic University of Najaf, College of Medical Technology, Department of Radiology.

² Kufa University, Faculty of Education for Girls, Kufa, Iraq.

ARTICLE INFO

Received: 22nd June 2026
Received in revised form: 24th
July 2026
Accepted: 29th July 2026
Published: 15th September
2026

*Corresponding Author:

Hanan A. Al-Haidari

E-mail :

hananahmed97@iunajaf.edu.iq

Citation : Al-Haidari , H. A, Abba, A. S, Al-khafaji , K. H, Ubaid, M. M, Waheed, G. M, & Abd, A. R. (2026). Radiologic Evaluation of the Upper Airway Before Anaesthesia. *Modern Journal of Health and Applied Sciences*, 3(2), 91-109.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a
[Creative Commons Attribution
Non-Commercial 4.0
International license](https://creativecommons.org/licenses/by-nc/4.0/).

ABSTRACT

Airway management is a critical pillar of an aesthetic and surgical practice. Any unforeseen airway obstruction during clinical procedures can lead to severe complications, including acute hypoxia or cardiac arrest. This study aims to highlight the pivotal role of advanced medical imaging as a diagnostic and proactive tool for evaluating the complex anatomy of the upper airway and predicting potential challenges during tracheal intubation. The study adopted an analytical approach by examining clinical cases from multiple medical centres, including Al-Sadr Teaching Hospital, Al-Najaf Teaching Hospital and the National Oncology Hospital. The sample encompassed various conditions, such as structural deformities, nasal septum deviation, and laryngeal tumours. The imaging protocols utilised included Computed Tomography (CT) for its superior visualisation of osseous structures, Magnetic Resonance Imaging (MRI) for precise soft-tissue characterisation, and conventional X-ray radiography. The findings indicate that integrating traditional clinical assessment with radiographic data effectively identifies obstructive factors, such as tonsillar hypertrophy, macroglossia, or airway narrowing associated with obesity. The study concludes that reliance on advanced imaging modalities significantly reduces the incidence of life-threatening events during anaesthesia, thereby enhancing patient safety standards and facilitating the development of precise contingency plans for difficult airway management.

Keywords: Radiologic, Anaesthesia, CT, MRI, X-ray, laryngitis, Hodgkin lymphoma.

1. Introduction

One of the most important components of effective airway management is orotracheal intubation, which involves choosing the appropriate tools and preparing different approaches to improve patient safety. Developments in medical anaesthetic and surgical procedures, such as airway blockage during procedures, may result in serious side effects like hypoxia, cardiac arrest, or arrhythmias, especially if the patient is completely dependent on anaesthetic assistance to ensure proper ventilation. Fibre-optic evaluation, computed tomography, and ultrasound of clinical evaluation using medical imaging are essential to prevent unanticipated, potentially fatal situations while under anaesthesia (Apfelbaum et al., 2022; Miller et al., 2020; RD, 2009). Preoperative airway assessment is essential for mitigating these risks by allowing medical professionals to determine the possibility of challenging mask ventilation, anomalies, and possible roadblocks using techniques like imaging. The use of imaging techniques has further enhanced the capacity to identify anatomical variations and abnormalities (Apfelbaum et al., 2022). These methods facilitate improved preoperative planning, lessen contemporary airway control, and lower anaesthesia-related issues (Walls & Murphy, 2008).

2. Background and Literature Review

Accurate anatomical assessment is fundamental to the diagnostic work-up and management of head and neck disease. In particular, familiarity with the magnetic resonance imaging (MRI) anatomy of the oral cavity and oropharynx is essential for evaluating lesions caused by neoplastic, infectious, inflammatory, or congenital disorders, because MRI offers excellent soft-tissue contrast and helps determine lesion extent and involvement of adjacent anatomical spaces (Tshering Vogel et al., 2010). In parallel, pre-anaesthetic assessment is evolving through the incorporation of structured clinical evaluation, imaging, digital tools, and risk-prediction approaches. However, the implementation of these emerging methods should remain grounded in the patient's immediate clinical condition, available resources, and perioperative realities (Viteri Hinojosa, 2024).

Imaging is also important in rhinology and endoscopic sinus surgery. In patients with sinonasal polyps, reduced expression of microRNA-29 has been associated with clinical manifestations such as nasal obstruction and with complete opacification of the anterior ethmoidal sinuses on computed tomography (CT), suggesting a potential relationship between miR-29 expression and disease severity (Ali et al., 2024). In addition, systematic interpretation of preoperative CT images is essential for identifying clinically important anatomical variations and disease extent before endoscopic sinus surgery. The CT checklist developed by Leong et al. helps standardise image interpretation, reduce overlooked findings, and support surgical planning (Leong et al., 2023).

Congenital airway abnormalities require early recognition because infants may present with stridor, abnormal cry, feeding difficulty, respiratory distress, or signs of airway obstruction. In such cases, awake flexible laryngoscopy is generally the first-line evaluation; direct laryngoscopy and bronchoscopy may be required when a more detailed assessment of the larynx, subglottis, trachea, or associated airway lesions is necessary (Varela & Schweiger, 2021). Point-of-care ultrasonography (POCUS) is a rapid, radiation-free adjunct for preoperative airway assessment and airway management. It can assist in predicting difficult intubation through measurements of anterior neck soft-tissue thickness, identify and mark the cricothyroid membrane before emergency front-of-neck access, and provide real-time confirmation of endotracheal-tube placement. However, airway ultrasound complements rather than replaces CT because ultrasound has limited assessment of bony structures and deep anatomical relationships (Lin et al., 2023).

3. General Physical Principles of Medical Imaging

This section discusses the basic physical principles of medical imaging and explains how these principles are applied in some medical imaging techniques.

3. 1. Principles of X-ray Imaging

X-rays are electromagnetic photons with high energy and a brief wavelength. Although they can enter soft tissues, they are heavily absorbed by dense substances like metal or bone. This variation in attenuation permits visualisation of internal structures such as teeth, implants, and bones. High-atomic-number agents, like barium or iodine, are used to see hollow organs and blood vessels. X-rays are produced in a vacuum tube, where electrons emitted from a heated cathode are accelerated toward a tungsten anode under high voltage. When electrons decelerate suddenly at the anode, braking radiation (Bremsstrahlung) is produced (Bushberg et al., 2020).

Characteristic X-rays are generated when inner-shell electrons of the target atom are displaced and replaced by higher-energy electrons. X-ray photons passing through the body may be transmitted, absorbed, or scattered. Absorption and scattering depend on tissue density and atomic number. Bone absorbs more radiation than soft tissue, resulting in brighter regions on radiographs. Scattered photons reduce image contrast and are partially removed using anti-scatter grids (Seeram, 2019; Sprawls, 1995).

3. 2. Computed Tomography (CT)

CT produces cross-sectional images of the body by measuring X-ray attenuation from multiple angles. Unlike conventional radiography, CT eliminates superimposition and provides depth information. A rotating X-ray tube and detector array acquire multiple projections around the patient. Computer algorithms reconstruct these projections into slices with high spatial resolution. Computed Tomography (CT) functions strictly as a diagnostic imaging modality rather than a therapeutic tool in the pre-anaesthetic evaluation of the upper airway. While CT does not deliver direct medical or surgical therapy, its diagnostic utility is critical for proactive intervention and contingency planning. By offering high-contrast, cross-sectional anatomical details, CT allows clinicians to detect subtle bone deformities, structural shifts, and soft-tissue masses that compromise airway space. Consequently, its diagnostic findings guide therapeutic and management decisions, such as modifying the anaesthetic approach, selecting specialised intubation equipment, or initiating surgical interventions (Brown et al., 2014).

3. 3. Magnetic Resonance Imaging (MRI)

MRI is based on the physical properties of protons (hydrogen nuclei) present in the human body, mainly in water and fat. Protons behave like tiny magnets because they possess spin and an associated magnetic moment. When the patient is placed inside a strong external magnetic field, most proton magnetic moments align either parallel or anti-parallel to the field (Al-Khafaji & Sahib, 2024). In the strong magnetic field of the MRI scanner, protons process around the direction of the magnetic field at a frequency known as the Larmor frequency, which is proportional to the magnetic field strength (42.6 MHz per Tesla for hydrogen). A short radiofrequency (RF) pulse is applied to disturb this alignment, tipping the protons away from equilibrium. When the RF pulse is switched off, the protons return to their original alignment while emitting radiofrequency signals that are detected by receiver coils. The return of protons to equilibrium occurs through two independent relaxation mechanisms:

- **T1 relaxation (longitudinal relaxation):** describes the rate at which protons lose excess energy and realign with the main magnetic field.
- **T2 relaxation (transverse relaxation):** describes the rate at which protons lose phase coherence due to local magnetic field differences. MRI images can be weighted based on proton density, T1 relaxation, or T2 relaxation, producing different tissue contrasts. MRI scanners typically operate at magnetic field strengths around 1.5–2 Tesla, which is much stronger than the Earth's magnetic field but not harmful to the human body. The main magnet is usually superconducting

and cooled with liquid helium to maintain 0 electrical resistance. Scan times can be as short as 0.5 seconds per slice, with slice thickness ranging from 0.5 to 2 mm (H Al-khafaji & A.Al-Haidari, 2025).

3. 4. Difference Between Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) in Upper Airway Assessment

Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) are complementary diagnostic tools, each offering distinct imaging capabilities for evaluating the upper airway (Al-Haidari & Al-khafaji, 2024):

a) Computed Tomography (CT):

- **Imaging Modality & Physics:** CT utilizes ionizing X-ray radiation to produce high-resolution, cross-sectional anatomical images.
- **Diagnostic Strengths:** CT is exceptional for visualizing osseous (bone) structures, calcifications, and acute airway emergencies.
- Clinical Advantages:** CT offers rapid acquisition time (completed in seconds), high spatial resolution, wide accessibility, lower cost, and is ideal for patients who cannot tolerate long scan durations or suffer from acute respiratory distress.
- **Limitations:** Exposure to ionizing radiation and limited soft-tissue contrast compared to MRI.

b) Magnetic Resonance Imaging (MRI):

- **Imaging Modality & Physics:** MRI utilises strong magnetic fields and non-ionising radiofrequency pulses.
- **Diagnostic Strengths:** MRI provides superior soft-tissue resolution, making it the gold standard for characterising mucosal lesions, laryngeal/pharyngeal tumours (e.g., Hodgkin lymphoma), and soft-tissue oedema.
- **Clinical Advantages:** MRI offers complete absence of ionising radiation. multi-planar capability with excellent contrast between tumour tissue and adjacent soft tissues.
- **Limitations:** Motion artifact susceptibility, long acquisition times, higher cost, limited availability, and contraindications in patients with metallic implants or severe claustrophobia.

4. Etiology of Perioperative Airway Obstruction

Airway obstruction during surgery can arise from a combination of anatomical, pathological, and anaesthetic-induced factors (H. Al-khafaji et al., 2026).

- **Anatomical and Traumatic Factors:** Skeletal disruptions, such as mandibular or nasal bone fractures, alter upper airway geometry and displace soft tissues posteriorly, thereby compromising airway patency.
- **Pathological and Neoplastic Factors:** Space-occupying lesions—including laryngeal tumours, laryngeal carcinomas, and Hodgkin lymphoma—directly compress or physically occlude the tracheobronchial tree.
- **Inflammatory and Oedematous Factors:** Inflammatory conditions, such as acute laryngitis or severe mucosal oedema, induce airway narrowing and markedly reduce the cross-sectional area of the airway.
- **Anaesthetic-Induced Loss of Muscle Tone:** General anaesthesia and neuromuscular blocking agents suppress upper airway muscle tone, such as the genioglossus muscle, allowing the tongue and soft palate to collapse against the posterior pharyngeal wall.

5. Data Collection and Processing

The clinical cases were obtained from Al-Sader Hospital, Al-Najaf Al-Ashraf Teaching Hospital and National Oncology Hospital with the approval of the Ministry of Health, in accordance with the official letter issued by the Islamic University/Technical College (No. 6799, dated 12/02/2026), which is attached at the end of this research. These cases were analysed using Microsoft Excel to extract statistical data and results. The work was carried out using a Dell computer.

6. Patient and Methods

Table 1. Al-Sader Hospital.

Name	Age	Sex	Weight	Diagnosis	Method Of Examination	Smoker	City
Case1	10	Male	32	Neoplasms of the Nasal Cavity and Larynx	MRI	NO	Najaf
Case2	35	Male	70	Mandibular Fracture	X_Ray	NO	Najaf
Case3	35	Male	70	Mandibular Fracture	CT	NO	Najaf
Case4	25	Male	64	Laryngitis	MRI	NO	Karbala
Case5	65	Male	76	Neoplasms of the Nasal Cavity and Larynx	MRI	NO	Najaf
Case6	18	Female	60	Laryngeal Cancer	MRI	NO	Diwaniya
Case7	26	Male	66	Deviated Septum	CT	Yes	Najaf
Case8	45	Male	82	Laryngeal Cancer	CT	NO	Najaf
Case9	43	Male	86	Laryngeal Cancer	CT	NO	Najaf
Case10	65	Female	90	Deviated Septum	CT	NO	Najaf
Case11	18	Male	70	Nasal Bone Fracture	CT	NO	Najaf
Case12	22	Male	72	Deviated Septum	CT	NO	Najaf
Case13	81	Male	96	Deviated Septum	CT	NO	Najaf
Case14	20	Male	73	Deviated Septum	CT	NO	Najaf
Case15	55	Male	87	Nasal Bone Fracture	CT	Yes	Najaf
Case16	65	Male	92	Deviated Septum	CT	NO	Najaf
Case17	24	Female	58	Deviated Septum	CT	NO	Najaf
Case18	31	Male	82	Neoplasms of the Nasal Cavity and Larynx	CT	Yes	Najaf
Case19	46	Female	71	Deviated Septum	CT	NO	Najaf
Case20	60	Male	88	Deviated Septum	CT	Yes	Najaf
Case21	18	Male	68	Deviated Septum	CT	NO	Najaf

Case22	21	Male	47	Nasal Bone Fracture	CT	NO	Najaf
Case23	35	Male	63	Nasal Bone Fracture	CT	NO	Najaf
Case24	48	Male	92	Laryngitis	CT	Yes	Najaf
Case25	57	Female	76	Nasal Bone Fracture	CT	NO	Najaf
Case26	66	Male	84	Nasal Bone Fracture	CT	Yes	Najaf
Case27	50	Male	84	Laryngeal Tumour	X_Ray	Yes	Diwaniya
Case28	50	Male	84	Laryngeal Tumour	CT	Yes	Diwaniya
Case29	50	Male	79	Laryngeal Cancer	MRI	NO	Najaf
Case30	75	Male	72	Deviated Septum	X_Ray	NO	Najaf
Case31	6	Male	20	Neoplasms of the Nasal Cavity and Larynx	MRI	NO	Bable
Case32	55	Female	73	Laryngitis	CT	NO	Najaf
Case33	55	Female	73	Laryngitis	MRI	NO	Najaf
Case34	15	Male	58	Neoplasms of the Nasal Cavity and Larynx	X_Ray	NO	Najaf
Case35	15	Male	58	Neoplasms of the Nasal Cavity and Larynx	MRI	NO	Najaf

Table 2. National Oncology Hospital.

Name	Age	Sex	Weight	Diagnosis	Method Of Examination	Smoker	City
Case36	90	Male	65	Deviated Septum	MRI	NO	Diwaniya
Case37	40	Female	68	Neoplasms of the Nasal Cavity and Larynx	CT	NO	Najaf
Case38	40	Female	68	Neoplasms of the Nasal Cavity and Larynx	MRI	NO	Najaf
Case39	13	Male	39	Mandibular Fracture	X_Ray	NO	Karbala
Case40	10	Male	35	Neoplasms of the Nasal Cavity and Larynx	CT	NO	Najaf
Case41	65	Male	97	Laryngitis	CT	Yes	Najaf
Case42	57	Male	91	Neoplasms of the Nasal Cavity and Larynx	CT	NO	Diwaniya
Case43	36	Male	72	Hodgkin Lymphoma	CT	Yes	Najaf
Case44	62	Female	88	Laryngeal Tumour	CT	NO	Diwaniya
Case45	85	Male	86	Hodgkin Lymphoma	CT	NO	Najaf
Case46	35	Female	75	Hodgkin Lymphoma	CT	NO	Najaf
Case47	26	Male	76	Laryngitis	CT	Yes	Najaf
Case48	20	Female	69	Hodgkin Lymphoma	CT	NO	Najaf

Case49	47	Male	77	Laryngitis	CT	NO	Najaf
Case50	25	Male	74	Hodgkin Lymphoma	CT	NO	Najaf
Case51	75	Female	87	Laryngitis	CT	NO	Najaf
Case52	60	Female	85	Laryngitis	CT	NO	Najaf
Case53	72	Male	70	Hodgkin Lymphoma	CT	NO	Najaf
Case54	61	Male	86	Hodgkin Lymphoma	CT	NO	Najaf
Case55	73	Female	81	Laryngitis	X-Ray	NO	Najaf
Case56	30	Male	84	Laryngitis	X-Ray	NO	Najaf
Case57	15	Female	53	Hodgkin Lymphoma	CT	NO	Najaf
Case58	60	Male	71	Neoplasms of the Nasal Cavity and Larynx	CT	NO	Babylon
Case59	55	Female	59	Laryngeal Cancer	MRI	NO	Najaf
Case60	72	Male	75	Laryngeal Cancer	CT	NO	Najaf

Table 3. Al-Najaf Teaching Hospital.

Name	Age	Sex	Weight	Diagnosis	Method Of Examination	Smoker	City
Case61	56	Female	63	Deviated Septum	CT	NO	Najaf
Case62	51	Female	81	Laryngitis	X-Ray	NO	Najaf
Case63	66	Female	78	Nasal Bone Fracture	CT	NO	Najaf
Case64	62	Female	73	Laryngeal Cancer	CT	NO	Najaf
Case65	70	Female	73	Laryngeal Cancer	CT	NO	Najaf
Case66	42	Male	82	Hodgkin Lymphoma	CT	Yes	Najaf
Case67	29	Female	65	Laryngitis	CT	NO	Najaf
Case68	55	Male	88	Laryngeal Tumour	CT	Yes	Diwaniya
Case69	31	Female	70	Hodgkin Lymphoma	CT	NO	Najaf
Case70	68	Male	74	Deviated Septum	CT	NO	Najaf
Case71	24	Male	77	Neoplasms of the Nasal Cavity and Larynx	CT	Yes	Diwaniya
Case72	50	Female	81	Laryngeal Cancer	X-RAY	Yes	Najaf
Case73	45	Male	93	Hodgkin Lymphoma	CT	Yes	Najaf
Case74	38	Female	68	Laryngeal Tumour	CT	NO	Najaf
Case75	63	Male	85	Nasal Bone Fracture	CT	Yes	Najaf

Case76	21	Male	72	Deviated Septum	CT	NO	Najaf
Case77	20	Female	75	Hodgkin Lymphoma	MRI	Yes	Najaf
Case78	25	Male	69	Hodgkin Lymphoma	MRI	Yes	Najaf
Case79	72	Female	74	Hodgkin Lymphoma	MRI	Yes	Najaf
Case80	61	Male	70	Hodgkin Lymphoma	MRI	Yes	Najaf
Case81	15	Female	86	Hodgkin Lymphoma	MRI	Yes	Najaf
Case82	42	Male	53	Hodgkin Lymphoma	X-RAY	Yes	Najaf
Case83	31	Female	82	Hodgkin Lymphoma	X-RAY	Yes	Najaf
Case84	45	Male	70	Hodgkin Lymphoma	X-RAY	Yes	Najaf
Case85	50	Male	55	Laryngeal Tumour	X_Ray	NO	Najaf
Case86	50	Male	50	Laryngeal Tumour	X_Ray	NO	Najaf
Case87	62	Female	52	Laryngeal Tumour	X_Ray	Yes	Najaf
Case88	55	Male	53	Laryngeal Tumour	MRI	Yes	Najaf
Case89	38	Female	56	Laryngeal Tumour	MRI	Yes	Najaf
Case90	55	Male	55	Laryngeal Tumour	MRI	Yes	Najaf
Case91	38	Female	54	Laryngeal Tumour	MRI	Yes	Najaf
Case92	75	Female	87	Laryngitis	MRI	Yes	Najaf
Case93	60	Female	85	Laryngitis	MRI	Yes	Najaf
Case94	73	Female	81	Laryngitis	MRI	Yes	Najaf
Case95	30	Male	84	Laryngitis	MRI	Yes	Najaf
Case96	51	Female	81	Laryngitis	MRI	Yes	Najaf
Case97	57	Female	47	Nasal Bone Fracture	X-RAY	NO	Najaf
Case98	66	Male	63	Nasal Bone Fracture	X-RAY	NO	Najaf
Case99	66	Female	76	Nasal Bone Fracture	X-RAY	NO	Najaf
Case100	63	Male	84	Nasal Bone Fracture	X-RAY	NO	Najaf
Case101	66	Female	78	Nasal Bone Fracture	X-RAY	NO	Najaf
Case102	63	Male	85	Nasal Bone Fracture	X-RAY	NO	Najaf
Case103	45	Male	60	Mandibular Fracture	X-Ray	NO	Najaf

Case104	45	Male	60	Mandibular Fracture	X-Ray	NO	Najaf
Case105	45	Male	60	Mandibular Fracture	X-Ray	NO	Najaf
Case106	46	Male	66	Mandibular Fracture	CT	NO	Najaf
Case107	49	Male	66	Mandibular Fracture	CT	NO	Najaf
Case108	50	Male	66	Mandibular Fracture	CT	NO	Najaf
Case109	51	Male	77	Mandibular Fracture	X-Ray	NO	Najaf

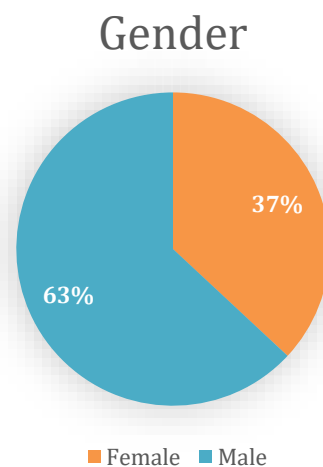


Figure 1. Percentage (%) of gender female and male.

Demographic analysis of the study sample revealed a higher prevalence and severity of upper airway pathologies among male patients (63%) compared to female patients (37%), as illustrated in **Figure 1**.

This gender variation can be attributed to several biomechanical, behavioural, and occupational factors:

1. **Trauma and Facial Fractures:** Male individuals generally exhibit higher exposure rates to high-energy facial trauma, road traffic accidents, and occupational hazards, leading to a higher incidence of complex nasal bone and mandibular fractures.
2. **Neoplastic Risk Factors:** High-risk behaviours, particularly chronic tobacco smoking and alcohol consumption, are statistically more prevalent among males in the studied demographic and directly contribute to an increased risk of laryngeal carcinoma and upper airway malignancies.
3. **Anatomical and Tissue Differences:** Males typically possess larger laryngeal dimensions and higher soft-tissue volume in the upper airway, which can lead to more pronounced airway narrowing and collapse under the influence of general anaesthetic agents.

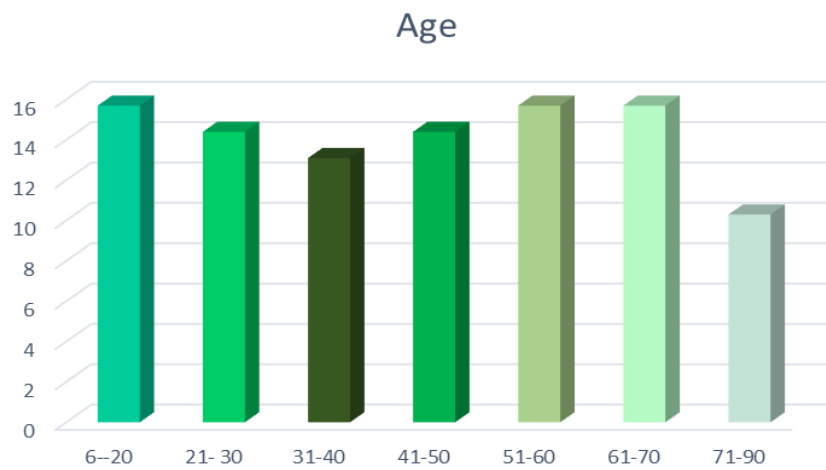


Figure 2. The mean of age among age groups.

This graph shows that the age groups between 6-20 and 50-90 are the most frequent visitors to hospitals.

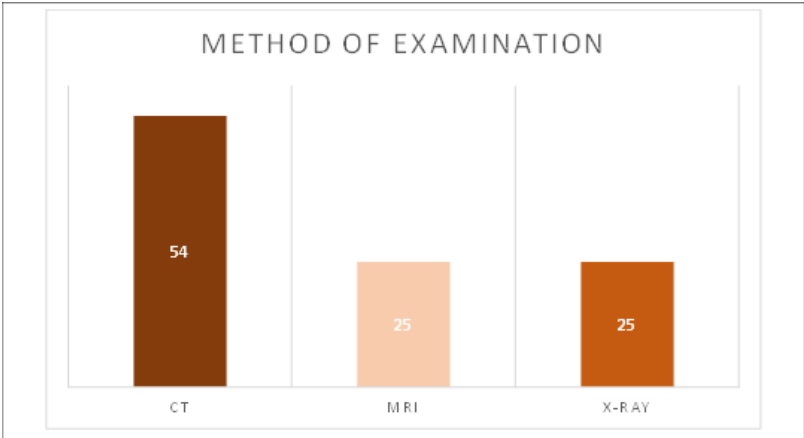


Figure 3. Method of examination.

This diagram shows us that most cases are examined using CT scans, and we attribute this to its accuracy, availability, speed of examination, and low cost.

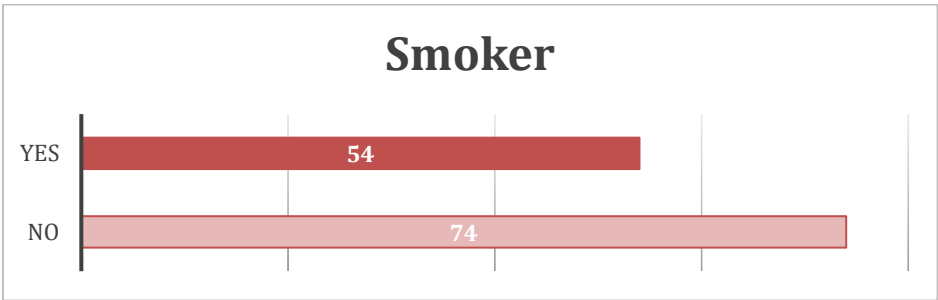


Figure 4. Percentage (%) of smokers.

This graph shows that the percentage of non-smoking patients is higher than the percentage of smoking patients.

Diagnosis

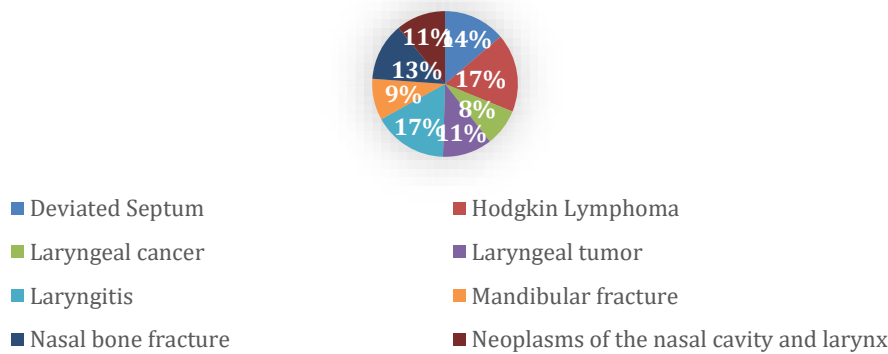


Figure 5. Percentage (%) of total number of disease cases.

This graph shows the percentage of diagnosed cases out of the total number of disease cases.

7. Results

Table 4. Shows the Cases Examined by X-ray.

Type of Condition	Total Number of Cases	Cases Detected by X-ray	Detection Rate (%)	Number of Cases in Which the Anaesthetic Plan Was Modified
Deviated Septum	16	1	6.25 %	1
Hodgkin Lymphoma	20	3	15 %	0
Laryngeal cancer	10	1	10 %	0
Laryngeal tumour	12	4	33.3 %	0
Laryngitis	18	3	16.7 %	0
Mandibular fracture	10	6	60 %	5
Nasal bone fracture	14	6	42.9 %	5
Neoplasms of the nasal cavity and larynx	12	1	8.3%	0

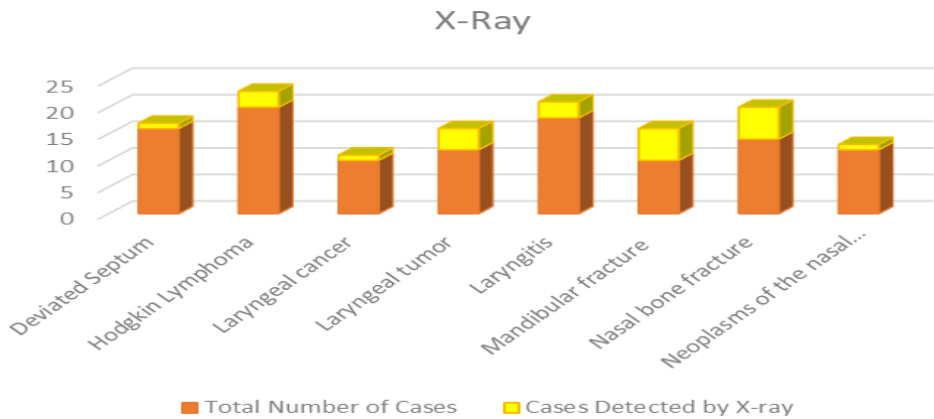


Figure 6. Cases Detected by X-Ray

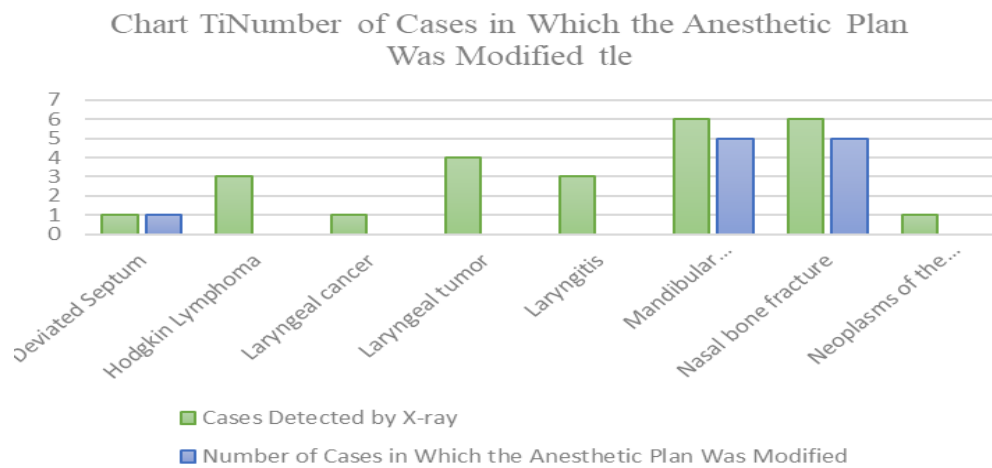


Figure 7. Number of Cases in Which the Anaesthetic Plan Was Modified by X-Ray.

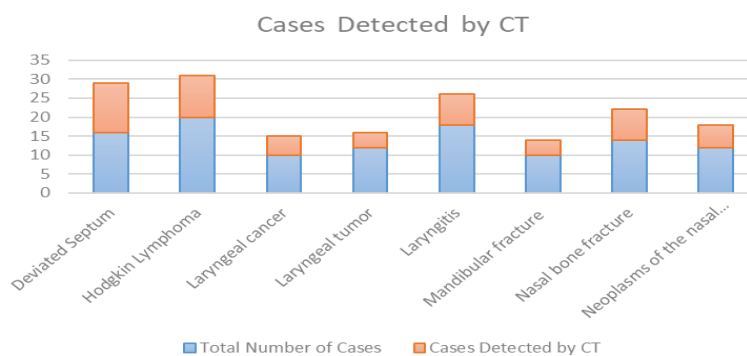


Figure 8. Cases Detected by CT.

Table 5. Shows the Cases Examined by CT.

Type of Condition	Total Number of Cases	Cases Detected by CT	Detection Rate (%)	N. Cases the Anaesthetic Plan Was Modified
Deviated Septum	16	13	81.25 %	10
Hodgkin Lymphoma	20	11	55 %	3
Laryngeal cancer	10	5	50 %	5
Laryngeal tumour	12	4	33.3 %	5
Laryngitis	18	8	44 %	8
Mandibular fracture	10	4	40 %	3
Nasal bone fracture	14	8	57.14 %	8
Neoplasms of the nasal cavity and larynx	12	6	50 %	5

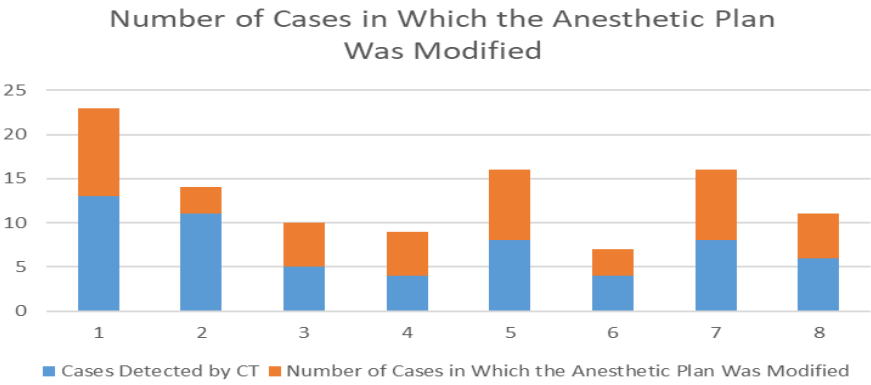


Figure 9. Number of Cases in Which the Anaesthetic Plan Was Modified CT.

The variance in the percentages of anaesthetic plan modifications depicted in Figure 9 reflects the distinct anatomical and pathological impacts of each condition on upper airway patency:

- 1. **High-Modification Pathologies:** Conditions presenting with severe anatomical narrowing, structural rigidity, or tissue mass such as laryngeal carcinomas, severe nasal septum deviations, and acute jaw/facial fractures demonstrated the highest modification rates (reaching up to 100%). These pathologies pose a direct threat of acute airway collapse during anaesthetic induction, necessitating proactive adjustments such as video laryngoscopy, awake fiberoptic intubation, or surgical tracheostomy preparation.
- 2. **Moderate- to Low-Modification Pathologies:** Conditions characterised by minor localised inflammation or non-obstructive tissue shifts required less frequent alterations to standard intubation protocols, as they posed a lower risk of complete airway occlusion.

Consequently, the degree of anaesthetic modification correlates directly with the structural severity and location of the lesion identified via CT imaging.

Table 6. Shows the Cases Examined by MRI.

Type of Condition	Total Number of Cases	Cases Detected by MRI	Detection Rate (%)	N. of Cases in Which the Anaesthetic Plan Was Modified
Deviated Septum	16	1	6.25	1
Hodgkin Lymphoma	20	5	25	4
Laryngeal cancer	10	3	30	1
Laryngeal tumour	12	4	33.3	1
Laryngitis	18	7	38.8	3
Mandibular fracture	10	4	40	3
Nasal bone fracture	14	0	0	0
Neoplasms of the nasal cavity and larynx	12	5	41.6	4

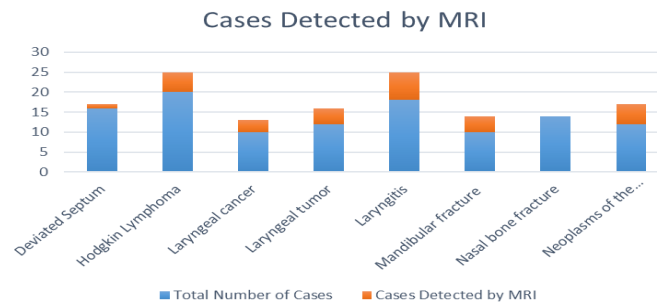


Figure 10. Cases Detected by MRI.

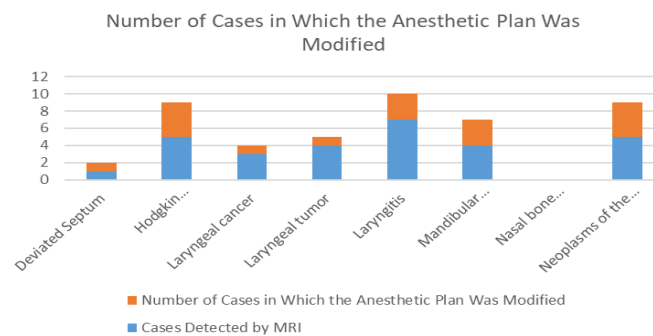


Figure 11. Number of Cases in Which the Anaesthetic Plan Was Modified by MRI.

Table 7. Shows Cases (Difficulty Airway) of Total Condition by X-RAY, CT, and MRI.

Imaging Modality	Total Cases Examined	Difficult Airway Diagnosed
X-ray (Plain Radiography)	25	11
CT scan (Computed Tomography)	59	47
MRI (Magnetic Resonance Imaging)	29	17

Although Magnetic Resonance Imaging (MRI) does not emit ionising radiation, Computed Tomography (CT) was utilised in the majority of clinical cases in this study (as shown in [Figure 3](#)). This clinical preference is justified by several critical factors in pre-anaesthetic airway evaluation:

- ✓ **Rapid Acquisition Time:** CT scans are completed within seconds, whereas MRI sequences require 20 to 45 minutes. For patients with compromised airways or acute dyspnoea, maintaining a supine position for extended periods during MRI poses a severe risk of acute asphyxiation.
- ✓ **Bony and Structural Detail:** CT provides superior spatial resolution for evaluating complex facial/nasal fractures and calcified laryngeal structures, which are essential for structural airway mapping.
- ✓ **Availability and Cost-Effectiveness:** CT scanners are widely available in emergency departments and teaching hospitals, with lower procedural costs and faster throughput compared to MRI.
- ✓ **Motion Artifact Reduction:** Because CT scanning is ultra-fast, it minimises motion artifacts caused by patient breathing, coughing, or distress, thereby ensuring reliable diagnostic images.

Consequently, the immediate diagnostic clarity and safety provided by CT during pre-anaesthetic planning outweigh the theoretical risks associated with ionising radiation in acute clinical settings.

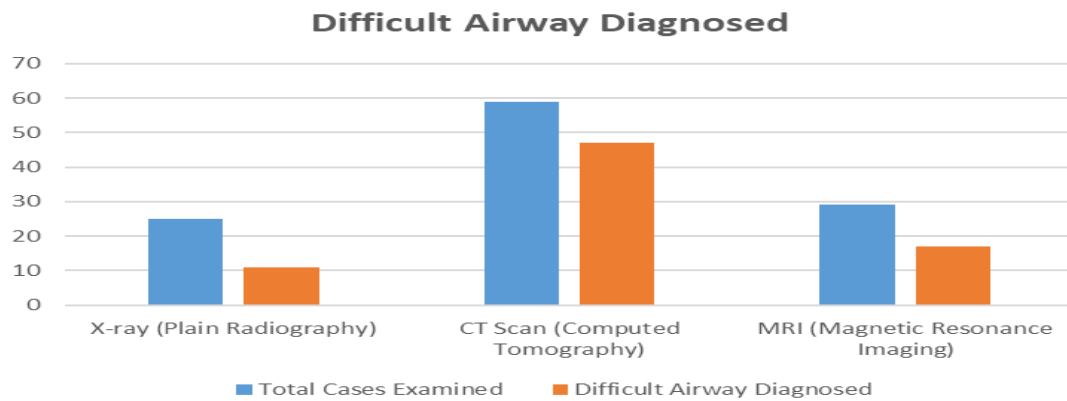


Figure 12. Difficult Airway Diagnosed.

8. Impact of Preoperative Imaging on Anaesthetic Management

Radiologic evaluation plays a pivotal role in modifying and tailoring anaesthetic strategy prior to surgery. By providing detailed anatomical maps of the upper airway, pre-anaesthetic medical imaging enables anaesthesiologists to identify potential airway compromise—such as subglottic stenosis, tracheal deviation, or laryngeal tissue mass—that may not be evident during routine bedside clinical exams (e.g., Mallampati scoring).

Consequently, these radiological findings directly influence anaesthetic management by:

- ❖ **Determining Intubation Strategy:** Guiding the decision between standard direct laryngoscopy and advanced techniques (e.g., video laryngoscopy, fiberoptic intubation, or awake intubation).
- ❖ **Selecting Airway Equipment:** Assisting in the precise sizing of endotracheal tubes to minimise mucosal trauma or airway resistance.
- ❖ **Emergency Preparedness:** Allowing the surgical and anaesthetic team to prepare for a "cannot intubate, cannot oxygenate" (CICO) scenario by having emergency surgical airway equipment (e.g., cricothyroidotomy kits) readily available.
- ❖ **Procedural Safety:** Notably, no catastrophic airway events, surgical airway emergencies, or complete intubation failures were recorded among patients evaluated with pre-anaesthetic imaging, reinforcing its critical role in clinical risk reduction.

9. Correlation of Pathological Diagnoses with Difficult Airway Mechanics

Although this investigation evaluated the diagnostic performance of cross-sectional imaging across specific clinical pathologies (e.g., maxillofacial fractures, soft-tissue masses, mucosal oedema, and tracheal deviation), these structural lesions serve as the primary pathological determinants of difficult airway management. Rather than relying exclusively on geometric or metric measurements, such as retropharyngeal space thickness or mandibular angle degree, the volumetric and morphological alterations delineated on CT and MRI—including luminal narrowing, anatomical displacement, and tissue rigidity—directly predict the physical difficulty encountered during laryngoscopy. Consequently, establishing a precise radiological diagnosis directly connects pre-procedural imaging findings with clinical airway difficulty scoring and proactive risk mitigation (Li et al., 2022).

10. Conclusion

- ✚ Study Limitations: A key limitation of this investigation is the relatively modest sample size ($n = 109$), which was gathered via convenience sampling across three major clinical centres. While this multi-centre approach enhances the representativeness of the findings, the study was designed as a descriptive pilot investigation focused on observing clinical imaging patterns and anaesthetic plan modifications, rather than establishing formal diagnostic test accuracy metrics, such as sensitivity, specificity, or predictive values. Consequently, subgroup observations across specific pathological categories should be interpreted as exploratory, warranting larger prospective trials to provide further statistical validation.
- ✚ **Table 4** presents the distribution of medical conditions examined using X-ray imaging, including the total number of cases for each condition, the number of cases detected by X-ray, the detection rate, and the impact of X-ray findings on modifications to the anaesthetic plan. The findings indicate that the effectiveness of X-ray imaging varies depending on the type of condition. It showed low diagnostic efficiency in certain conditions, such as deviated septum, where only 1 out of 16 cases was detected (6.25%), as well as in neoplasms of the nasal cavity and larynx, with a detection rate of 8.3%. Similarly, detection rates were low to moderate in conditions such as Hodgkin lymphoma (15%), laryngeal cancer (10%), and laryngitis (16.7%), while relatively better detection was observed in laryngeal tumors, reaching 33.3%.
- ✚ In contrast, X-ray imaging demonstrated high effectiveness in diagnosing bony injuries. The highest detection rate was observed in mandibular fractures (60%), followed by nasal bone fractures (42.9%), indicating that X-ray is a reliable diagnostic tool for such conditions. Regarding the impact on the anaesthetic plan, modifications were minimal in most cases, except in fracture-related conditions. The anaesthetic plan was modified in 5 cases of mandibular fractures and 5 cases of nasal bone fractures, as well as in one case of deviated septum. This highlights the clinical importance of X-ray findings in guiding anaesthetic decisions, particularly in cases involving bony injuries. Overall, it can be concluded that X-ray imaging has high diagnostic value in detecting fractures, while its effectiveness is limited in diagnosing tumours and inflammatory conditions, thus necessitating the use of additional diagnostic modalities in such cases, as in **Figures 6 and 7**.
- ✚ **Table 5** presents the results of using computed tomography (CT) in the diagnosis of various medical conditions, highlighting its detection efficiency as well as its impact on modifying the anaesthetic plan. It displays the total number of cases for each condition, the number of CT-detected cases, and the matching rate of detection. The results show that CT demonstrated the greatest diagnostic precision in deviated septum cases, with a detection rate of 81.25%, as 13 of the 16 cases were found. Meanwhile, a number of laryngeal cancers and neoplasms of the nasal cavity and larynx had detection rates of about 50%, whereas conditions like nasal bone fractures (57.14%) and Hodgkin lymphoma (55%) had moderate detection rates. Lower detection rates were observed in laryngeal tumours (33.3%), mandibular fractures (40%), and laryngitis (44%), suggesting limited sensitivity of CT in these conditions.
- ✚ Furthermore, the table highlights the important role of CT in influencing anaesthetic management. A considerable number of cases required modification of the anaesthetic plan based on CT findings, particularly in patients with a deviated septum (10 cases) and those with laryngitis and nasal bone fractures (8 cases each). On the other hand, CT had a lesser impact on anaesthetic decisions in conditions such as Hodgkin lymphoma and mandibular fractures, where only 3 cases required modification. Overall, CT can be considered a valuable diagnostic tool; however, its effectiveness varies depending on the type of condition. Additionally, it plays a significant role in improving anaesthetic decision-making, thereby enhancing patient safety during medical procedures, as illustrated in **Figures 8 and 9**.
- ✚ **Table 6** presents the results of using MRI in diagnosing a variety of medical conditions, as well as its impact on modifying the anaesthetic plan. The findings show that the effectiveness of MRI varies depending on the type of condition. In cases of a deviated septum, MRI had a very limited role, detecting only 1 out of 16 cases, which represents

a low detection rate. In contrast, for Hodgkin lymphoma, MRI demonstrated a relatively better performance, identifying 5 out of 20 cases, and contributing to changes in the anaesthetic plan in several instances.

- ✚ Regarding laryngeal conditions, such as laryngeal cancer and laryngeal tumours, MRI showed moderate effectiveness, with detection rates ranging from 30% to 33.3%. Its performance was slightly higher in cases of laryngitis, where the detection rate reached approximately 38.8%.
- ✚ For fracture-related conditions, MRI proved useful in detecting mandibular fractures, with a detection rate of 40%. However, it showed no effectiveness in nasal bone fractures, as no cases were detected.
- ✚ The highest detection rate was observed in neoplasms of the nasal cavity and arynx, reaching 41.6%. These cases also had a notable impact on modifying the anaesthetic plan.
- ✚ Overall, MRI appears to be more effective in diagnosing tumours and inflammatory conditions than simple fractures, and it plays an important role in guiding clinicians to adjust anaesthetic plans according to the patient's condition , as shown in [Figures 10 and 11](#).
- ✚ **Table 7** presents the number of cases examined using three different imaging modalities X-ray (plain radiography), CT (computed tomography), and MRI (magnetic resonance imaging) along with the number of cases diagnosed as “difficult airway” by each method.
- ✚ Overall, it can be observed that the highest number of examined cases was with CT, totalling 59 cases, followed by MRI with 29 cases and X-ray with 25 cases. In terms of diagnosed difficult airway cases, CT also recorded the highest number at 47 cases, indicating a greater ability to detect this condition compared to the other modalities.
- ✚ In contrast, X-rays diagnosed only 11 cases out of 25, which is relatively low and may reflect limited sensitivity in detecting difficult airways. MRI diagnosed 17 cases out of 29, showing a moderate performance between CT and X-ray.
- ✚ Additionally, CT is more commonly used in emergency situations and urgent procedures than MRI because CT scans are faster and provide results in a short time, which is critical in life-threatening or time-sensitive conditions. In comparison, MRI is relatively slower and requires more time to complete, making it less suitable for emergency use. Overall, CT appears to be the most effective imaging modality for diagnosing a difficult airway, particularly in emergency settings, followed by MRI, while X-ray ranks last in terms of diagnostic capability, as in [Figure 12](#).

Conflict of Interest

The authors declare that there is no conflict of interest or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

All necessary data generated or analyzed during this study are included in this article. Any additional data could be available from the corresponding author upon request.

References

- Al-Haidari, H. A., & Al-khafaji, K. H. (2024). Studying the Effect of the Primary Coefficients (X, U) of the Logistic Chaotic Encryption Algorithm Equation on the Performance of X-Ray Medical Image Encryption. *Sebha University Conference Proceedings*, 134–139. <http://www.sebhau.edu.ly/journal/sucp/article/view/3293>
- Ali, M. M. M., Guindi, S. S., Qotb, M., Ibrahim, M. M., Gaber, S. N., & Abdelaleem, O. O. (2024). Computed Tomography (CT) scan of the paranasal sinuses and microRNA expression in patients with Nasal polypi.. *Fayoum University Medical Journal*, 14(1), 131–141. <https://doi.org/10.21608/fumj.2024.269412.1322>
- Al-Khafaji, K. H., & Sahib, H. A. (2024). Designing a Digital System for Enhancing, Coloring, Encryption and Decryption of X-ray Medical Image. 2024 15th International Conference on Information and Communication Systems (ICICS), 1–6. <https://doi.org/10.1109/ICICS63486.2024.10638292>
- Apfelbaum, J. L., Hagberg, C. A., Connis, R. T., Abdelmalak, B. B., Agarkar, M., Dutton, R. P., Fiadjoe, J. E., Greif, R., Klock, P. A., Mercier, D., Myatra, S. N., O'Sullivan, E. P., Rosenblatt, W. H., Sorbello, M., & Tung, A. (2022). 2022 American Society of Anesthesiologists Practice Guidelines for Management of the Difficult Airway *. *Anesthesiology*, 136(1), 31–81. <https://doi.org/10.1097/ALN.0000000000004002>
- Brown, R. W., Cheng, Y.-C. N., Haacke, E. M., Thompson, M. R., & Venkatesan, R. (2014). *Magnetic Resonance Imaging: Physical Principles and Sequence Design*. John Wiley & Sons.
- Bushberg, J. T., Seiberg, J. A., Jr, E. M. L., & Boone, J. M. (2020). *The Essential Physics of Medical Imaging*. Wolters Kluwer Health.
- H Al-khafaji, K., & A.Al-Haidari, H. A. A.-H. (2025). Gray X-Ray medical images encryption and decryption by chaotic algorithm. *Modern Journal of Health and Applied Sciences*, 2(1), 1–11. <https://doi.org/10.70411/MJHAS.2.1.2025143>
- H. Al-khafaji, K., A.Al-Haidari, H., & Mohammed Mashkoor, Z. (2026). MRI Images Colorization and Encryption Using 2RGB and Chaotic Maps. *Modern Journal of Health and Applied Sciences*, 3(1), 18–28. <https://doi.org/10.70411/MJHAS.3.1.2026247>
- Leong, S., Yang, N., Ray, A., Akbar, N., Colley, P. M., Signore, A. D., Eloy, J. A., Govindaraj, S., Gudis, D. A., Helman, S., Hsueh, W., Iloreta, A., Kacker, A., Lieberman, S., Pearlman, A. N., Schaberg, M. R., Tabaei, A., Overdevest, J. B., & New York Rhinology Consortium. (2023). Development of an optimized preoperative computed tomography imaging checklist for endoscopic sinus surgery utilizing a systematic review of the literature and the modified Delphi method. *International Forum of Allergy & Rhinology*, 13(3), 196–204. <https://doi.org/10.1002/alr.23064>
- Li, X., An, B., Jiang, B., Xu, S., Liu, H., & Zhao, H. (2022). Pharynx volume derived from three-dimensional computed tomography is associated with difficult intubation in spinal deformity surgery: A retrospective cohort study. *Medicine*, 101(41), e31139. <https://doi.org/10.1097/MD.00000000000031139>
- Lin, J., Bellinger, R., Shedd, A., Wolfshohl, J., Walker, J., & Healy, J. (2023). Point-of-care ultrasound in airway evaluation and management. *A Comprehensive Review*, 13, 1541.
- Miller, R. D., Cohen, N. H., Eriksson, L. I., Fleisher, L. A., Wiener-Kronish, J. P., & Young, W. L. (2020). *Vascular access and monitoring*. *Miller's Anesthesia*. 9th Ed. Philadelphia: Elsevier.
- RD, M. (2009). Airway management in the adult. *Miller's Anesthesia*, 2, 1573–1576.
- Seeram, E. (2019). Digital Radiography: An Overview. In E. Seeram, *Digital Radiography* (pp. 1–19). Springer Singapore. https://doi.org/10.1007/978-981-13-3244-9_1
- Sprawls, P. (1995). *Physical principles of medical imaging*. Medical Physics Pub. Madison. <http://www.just.edu.jo/FacultiesandDepartments/FacultyofScienceandArts/Departments/AppliedPhysicalSciences/Documents/PHY%20783.pdf>

- Tshering Vogel, D. W., Zbaeren, P., & Thoeny, H. C. (2010). Cancer of the oral cavity and oropharynx. *Cancer Imaging*, 10(1). <https://doi.org/10.1102/1470-7330.2010.0008>
- Varela, P., & Schweiger, C. (2021). Congenital airway anomalies. *Seminars in Pediatric Surgery*, 30(3), 151055. <https://doi.org/10.1016/j.sempedsurg.2021.151055>
- Viteri Hinojosa, A. S. (2024). Emerging Trends in Pre-Anesthetic Assessment: A Systematic Review of Novel Approaches and Technologies. *Metro Ciencia*, 32(3), 35–42. <https://doi.org/10.47464/MetroCiencia/vol32/3/2024/35-42>
- Walls, R. M., & Murphy, M. F. (2008). *Manual of Emergency Airway Management*. Lippincott Williams & Wilkins.



A Conceptual Framework for Relativistic Neuroscience: Temporal Layer Decoupling During Extreme Time Dilation

Abdulwahhab A. Elkuwafi¹, Mohamed Mahmoud Mohsen¹, Aziza Al-Tarhouni^{2*}, Salleem F. Salleem³



¹Department of Therapeutic and Diagnostic Radiology, College of Medical Technology, Benghazi, Libya.

²Department of Medical Engineering, College of Medical Technology, Benghazi, Libya

³Department of Physics, College of Science, Al-Bidah, Libya.

ARTICLE INFO

ABSTRACT

Received: 19th May 2026

Received in revised form: 23rd July 2026

Accepted: 31th July 2026

Published: 15th September 2026

*Corresponding Author:

Aziza Al-Tarhouni

E-mail :

azizaatarhoni173@gmail.com

Citation : Elkuwafi, A. A, Mohsen, M. M, Al-Tarhouni , A. & Salleem, S. F. (2026). A Conceptual Framework for Relativistic Neuroscience: Temporal Layer Decoupling During Extreme Time Dilation. *Modern Journal of Health and Applied Sciences*, 3(2), 110-124.

Copyright (c) 2026 Modern Journal of Health and Applied Sciences (MJHAS)

Open Access



This work is licensed under a [Creative Commons Attribution Non Commercial 4.0 International license](https://creativecommons.org/licenses/by-nc/4.0/).

The personal experience of time during extreme perspective-dependent conditions remains poorly understood at the convergence of physics and neuroscience. We present an abstract framework that differentiates four temporal variables external coordinate time, traveller proper time, neural execution time, and perceptual time to analyse the conditions necessary for conscious experience during high-velocity transit. Our analysis exemplifies that, while special relativity forecasts dramatic time dilation in the traveller's frame, all biological processes remain constrained by the traveller's proper time. We derive the minimum proper time threshold (~50–100 ms) required for conscious apprehension based on neural dynamics fluctuations and show that near-light-speed travel over interstellar distances can provide adequate proper time for rich individualised experiences, whereas certain spacetime continuum shortcuts may not. We connect this paradigm to terrestrial analogues in neuropsychiatric pathologies, where temporal processing layers decouple, and propose biomedical engineering approaches for consciousness monitoring during future astronautics. The framework generates testable projections through computational neuroscience simulations, virtual reality projections, and patient studies. We explicitly acknowledge the risky nature of wormhole applications and the dearth of direct experimental validation for relativistic consciousness studies.

Keywords: Relativistic neuroscience, Time dilation, Proper time, Neural oscillatory dynamics, Consciousness monitoring.

DOI: <https://doi.org/10.70411/MJHAS.3.2.2026406>

1. Introduction

1. 1. The Problem of Time in Relativistic and Biological Frames

Time is conceived fundamentally differently across academic disciplines. In classical Newtonian physics, time was deemed absolute and universal (Newton, 1687). Einstein's theories of distinctive (Einstein, 1905) and general (Einstein, 1916) relativity founded that time is relative, observer-dependent, and closely linked with space in a four-dimensional spectrum. Under relativistic conditions particularly at velocities approaching the speed of light ($c \approx 3 \times 10^8$ m/s) or in strong gravitational fields time dilates according to the electromagnetic transformation.

Simultaneously, neurobiology has revealed that the brain's awareness of time is neither absolute nor directly associated with physical clock time (Eagleman, 2008; Wittmann, 2013). Humans exhibit methodical temporal distortions: exaggeration during novel or intimidating experiences, understatement during flow states, and biases in reflective judgements (Wittmann, 2013). The neural mechanisms underlying temporal processing involve distributed networks spanning the basal ganglia, cerebellum, prefrontal cortex, and parietal regions (Buhusi & Meck, 2005; Merchant et al., 2013). Neural signal transmission occurs at velocities ranging from 0.5 to 120 m/s, roughly six to nine orders of magnitude slower than light (Purves et al., 2001).

A conceptual question appears when these domains intersect: What are the necessary and adequate conditions for conscious experience during drastic relativistic travel? According to special relativity, as velocity approaches c , the Lorentz factor $\gamma = [1 - (v/c)^2]^{-1/2}$, grows without bound. For a traveller moving at $v = 0.999999c$, $\gamma \approx 707$, meaning that one year of coordinate time matches to approximately 12.4 hours of proper time. From the traveller's perspective, their own neural processes proceed normally; there is no contradiction within the traveller's rest frame. However, the extreme condensation of proper time relative to coordinate time raises questions about the minimum proper time required to sustain conscious awareness and whether certain transit scenarios, particularly those involving spacetime shortcuts, might fall below this threshold.

1. 2. Current Gaps in the Literature

Despite extensive research in relativistic physics and neuroscience, integrated models addressing this junction remain sparse. Physics literature focuses on the mathematical formalism of time dilation and spacetime geometry (Carroll, 2004; Misner et al., 1973), while neuroscience investigates temporal perception primarily under terrestrial conditions (Ivry & Schlerf, 2008; Matthews & Meck, 2016). Recent work has explored how altered gravitational fields affect neural function (Clément & Reschke, 2008; Manzey & Lorenz, 1998), and there is growing interest in cognitive challenges during extended spaceflight (Van Ombergen et al., 2017; Zivi et al., 2026). However, no comprehensive framework currently:

- ❖ Formally differentiates between coordinate, proper, neural, and sensory time under relativistic conditions;
- ❖ Derives minimum proper time boundaries for consciousness from neurobiological constraints;
- ❖ Connects relativistic time dilation to known mechanisms of neural temporal processing;
- ❖ Proposes biomedical engineering protocols for tracking temporal consciousness during space travel.

1. 3. Objectives and Scope

This paper proposes a conceptual framework differentiating four temporal layers: External Coordinate Time (T_{coord}), Traveller Proper Time (T_{proper}), Neural Processing Time (T_{neuro}), and Perceptual Time (T_{percept}). Our central hypothesis states that during extreme relativistic events, these layers exhibit relative disconnecting: while T_{coord} and T_{proper} diverge according to benchmark relativistic equations, T_{neuro} remains constrained by T_{proper} , and T_{percept} emerges as a cognitive construction modulated by attention, memory, and affective state.

We directly note that this framework is theoretical and hypothetical. It does not claim to "resolve" any paradox (as none exists within standard special relativity), nor does it assert experimental validation. Rather, it provides a structured approach to

asking testable questions about consciousness under conditions that may eventually be encountered during interstellar spaceflight.

The rest of this paper is organised as follows: Section 2 develops the theoretical framework for our temporal model. Section 3 presents the integrated biophysical analysis and applies it to transit scenarios. Section 4 discusses the implications for neuroscience, space travel, and biomedical engineering, including constraints. Section 5 provides finalizing remarks.

2. Theoretical Framework: A Multi-Layered Model of Time

2. 1. Physical Time: Coordinate and Proper Time

2. 1. 1. Special Relativistic Time Dilation

Physical time in relativity is not a single quantity; rather, it must be carefully designated with respect to an observer's reference frame. Coordinate time (t or T_{coord}) is the time measured by a clock in an inertial reference frame (e.g., Earth). Proper time (τ or T_{proper}) is the invariant time measured by a clock moving with the traveller along its worldline.

For an object moving at constant velocity v relative to a stationary inertial observer, the relationship between coordinate time interval Δt and proper time interval $\Delta \tau$ is given by the Lorentz transformation (Einstein, 1905).

$$\Delta \tau = \Delta t \sqrt{1 - \frac{v^2}{c^2}} = \frac{\Delta t}{\gamma} \quad (1)$$

where the Lorentz factor is:

$$\gamma = \frac{1}{\sqrt{1 - \frac{v^2}{c^2}}} \quad (2)$$

Equation (1) reveals that as $v \rightarrow c$, the proper time experienced by the traveller approaches zero for any finite coordinate time interval. Table 1 illustrates this relationship across various velocities.

Table 1. Time dilation at various velocities. The coordinate time interval is fixed at $\Delta t = 1$ year (Earth observer).

v/c	γ	$\Delta \tau / \Delta t$	$\Delta \tau$ (traveller)
0.5	1.155	0.866	10.4 months
0.9	2.294	0.436	5.2 months
0.99	7.089	0.141	1.7 months
0.999	22.366	0.045	16.3 days
0.9999	70.712	0.014	5.2 days
0.99999	223.607	0.0045	1.6 days
0.999999	707.107	0.0014	12.4 hours

Note that at $v = 0.999999c$, the Lorentz factor is $\gamma \approx 707$ (not 22,360, as might be obtained by miscounting nines). This value is used in all subsequent calculations. The nonlinear growth of the Lorentz factor with velocity is illustrated in [Figure 1](#).

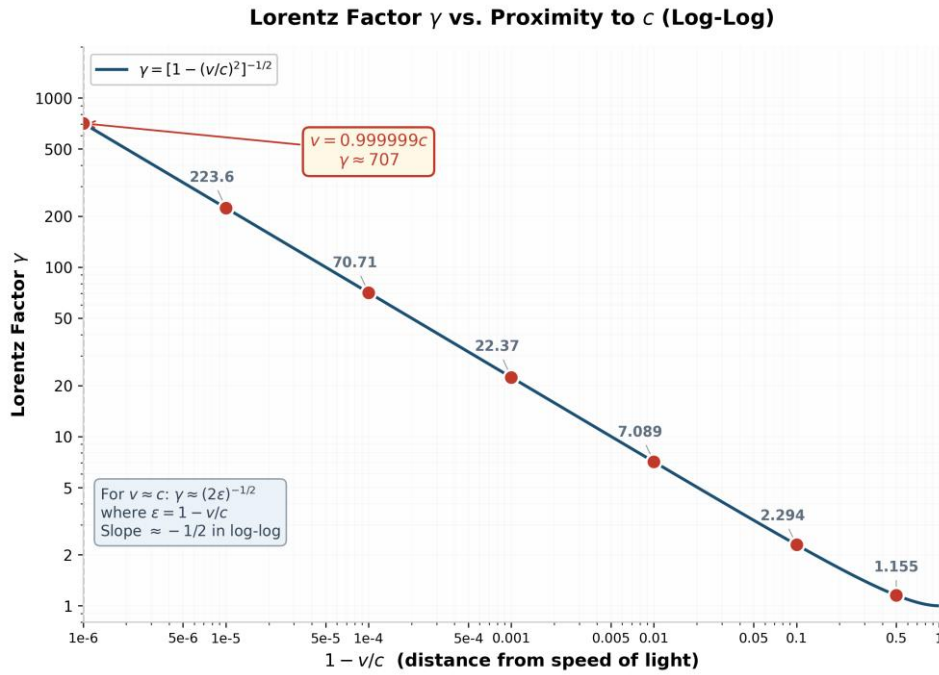


Figure 1. Lorentz factor γ as a function of nearness to the speed of light, plotted as γ versus $1 - v/c$ on logarithmic axes.

The linear behaviour in log-log space reveals the approaching power-law scaling $\gamma \sim (2\epsilon)^{(-1/2)}$ for $\epsilon = 1 - v/c \ll 1$. General relativity expands this framework to include gravitative time dilation. Near a massive object, the Schwarzschild metric gives:

$$d\tau = dt \sqrt{1 - \frac{2GM}{rc^2}} \quad (3)$$

where G is the gravitative constant, M is the mass, and r is the radial coordinate. Near the event horizon ($r \rightarrow 2GM/c^2$), proper time expansion becomes infinite relative to coordinate time.

2. 1. 2. Wormholes and Spacetime Shortcuts: A Boundary Condition Analysis

Morris and Thorne (1988) Proven that Einstein's field equations permit crossable wormhole solutions (Morris & Thorne, 1988). The proper distance l along a wormhole throat introduces a transit time:

$$T_{\text{proper}} \approx \frac{l}{v_{\text{traverse}}} \quad (4)$$

For a throat of length $l = 100$ m traversed at $v = 0.99c$, the proper time is approximately 337 ns. This duration is $\sim 7.4 \times 10^4$ times shorter than the ~ 25 ms period of a gamma oscillation, which is the minimum temporal unit of aware perception. Consequently, such traversal would provide inadequate proper time for neural processing, and we speculate that conscious experience could not be prolonged during the transit itself.

We highlight that wormhole physics remains entirely hypothetical. We include it not as a realistic situation, but as a limiting condition analysis to exemplify how our framework differentiates between transit situations that do and do not permit conscious experience.

2. 2. Neural Processing Time: Biological Constraints

Neural processing time (T_{neuro}) represents the time required for neural signals to propagate and for the brain to integrate information. Unlike coordinate time, T_{neuro} is not an independent temporal dimension but unfolds within the traveller's proper time. All neural processes—including action potentials, synaptic conveyance, and oscillatory dynamics—experience identical relativistic effects as the rest of the traveller's body. From the traveller's viewpoint, neural activity proceeds at its characteristic biological rate; there is no “extra” processing time available beyond T_{proper} .

2. 2. 1. Neural Signal Propagation

Action potentials propagate along axons at velocities determined by diameter and myelination. Transmission velocity can be approximated by:

$$v_{\text{neuro}} = k \cdot d \quad (5)$$

where d is the axon diameter and $k \approx 6 \text{ m/s}/\mu\text{m}$ for myelinated fibres (Purves et al., 2001). In humans, velocities range from 0.5-2 m/s (unmyelinated C fibres) to 70-120 m/s (myelinated A α fibres). The fastest signals travel at approximately 120 m/s- 2.5×10^6 times slower than light.

2. 2. 2. Synaptic and Network Delays

Each synapse introduces a delay of approximately 0.5 – 2 ms due to neurotransmitter release, diffusion, and receptor binding. For a neural circuit involving N_s synapses with an average delay τ_{syn} , the total synaptic transmission time is:

$$T_{\text{syn}} = N_s \cdot \tau_{\text{syn}} \quad (6)$$

Complex cognitive processes may involve dozens to hundreds of synaptic steps, creating processing requirements of tens to hundreds of milliseconds.

2. 2. 3. Neural Oscillations and the Temporal Window of Consciousness

The brain processes information through synchronised oscillations at characteristic frequencies (Buzsáki & Draguhn, 2004):

- Delta (0.5 - 4 Hz): Deep sleep
- Theta (4 - 8 Hz): Memory encoding
- Alpha (8 - 13 Hz): Sensory inhibition
- Beta (13 - 30 Hz): Active cognition
- Gamma (30 - 100 Hz): Conscious perception, binding

Gamma oscillations (30 - 100 Hz) are particularly relevant, as they represent the smallest temporal window associated with conscious perception, with a period of 10 - 33 ms (Pöppel, 2009). We propose that a minimum proper time threshold for conscious experience is:

$$T_{\text{proper}}^{\text{min}} \approx 2 - 4 \times T_\gamma \approx 50 - 100 \text{ ms} \quad (7)$$

This corresponds to 2 – 4 gamma cycles, allowing for minimal perceptual integration and temporal binding (Pöppel, 1997).

If $T_{\text{proper}} < T_{\text{proper}}^{(\text{min})}$, the model predicts that conscious experience cannot be sustained.

2. 2. 4. Mathematical Model and the Proper Time Constraint

For a sequence of N_e cognitive events, each requiring pathway length L_i , signal velocity v_{neuro} , S_i synaptic steps, and processing time $\tau_{\text{proc},i}$, the total neural processing time is:

$$T_{\text{neuro}} = \sum_{i=1}^{N_e} \left(\frac{L_i}{v_{\text{neuro}}} + (S_i \times \tau_{\text{syn}}) + \tau_{\text{proc},i} \right) \quad (8)$$

Critically, all neural processing is constrained by the available proper time:

$$T_{\text{neuro}} \leq T_{\text{proper}} \quad (9)$$

This inequality reflects the fundamental physical reality that neural computation cannot exceed the invariant time along the traveller's worldline. The brain's massively parallel architecture allows substantial computation within a given proper time interval, but it cannot create time that does not exist in the traveller's frame.

2. 3. Perceptual Time: The Subjective Construction

Perceptual time (T_{percept}) represents the subjective, conscious experience of duration. It is not a direct readout of physical time but a cognitive construction influenced by attention, emotional arousal, memory encoding, and cognitive load (Block et al., 2010; Droit-Volet & Meck, 2007; Ornstein, 1975; Tse et al., 2004).

2. 3. 1. Neural Correlates and the Internal Clock Model

Functional neuroimaging identifies key regions in temporal processing (Coull et al., 2004; Wiener et al., 2010), including the basal ganglia (interval timing), cerebellum (millisecond timing), prefrontal cortex (working memory for temporal information), and parietal cortex (spatial-temporal integration). The "internal clock" model proposes a pacemaker-accumulator mechanism where subjective duration depends on pulse accumulation (Gibbon et al., 1984).

2. 3. 2. Pathological Decoupling: Terrestrial Analogues

Temporal perception abnormalities in neuropsychiatric conditions provide evidence for the disposability of T_{neuro} and T_{percept} :

- ❖ **ADHD:** Consistent time underestimation and impaired temporal foresight (Barkley et al., 1997), suggesting accumulator dysfunction.
- ❖ **Depression:** Subjective time slowing (Kitamura & Kumar, 1982), possibly reflecting increased attention to internal states.
- ❖ **Schizophrenia:** Deficits in temporal order judgement and duration estimation (Volz et al., 2001), associated with disrupted gamma and theta oscillations.
- ❖ **Parkinson's disease:** Impaired interval timing following dopaminergic degeneration (Malapani et al., 1998).

We emphasise that these disorders involve biochemical, oscillatory, and attentional mechanisms—not relativistic effects. They serve as terrestrial analogues illustrating how $T_{\text{neuro}} \rightarrow T_{\text{percept}}$ decoupling can occur, but not as direct equivalents to relativistic scenarios.

2. 3. 3. Phenomenological Model

We represent T_{percept} as a transformation of T_{neuro} modulated by cognitive state:

$$T_{\text{percept}} = f(T_{\text{neuro}}; \alpha, \epsilon, \mu, \chi) \quad (10)$$

where α (attention), ϵ (emotional arousal), μ (memory encoding), and χ (cognitive load) are dimensionless modulation factors. A simplified linear approximation is:

$$T_{\text{percept}} \approx \alpha \cdot \epsilon \cdot \mu \cdot \chi \cdot T_{\text{neuro}} \quad (11)$$

Under normal conditions, $\langle \alpha \epsilon \mu \chi \rangle \approx 1$, giving $T_{\text{percept}} \approx T_{\text{neuro}}$. Extreme states can produce significant deviations. **Figure 2** illustrates how the ratio $T_{\text{percept}}/T_{\text{neuro}}$ varies with attention and affective arousal under normal retention and cognitive load conditions ($\mu = \chi = 1.0$).

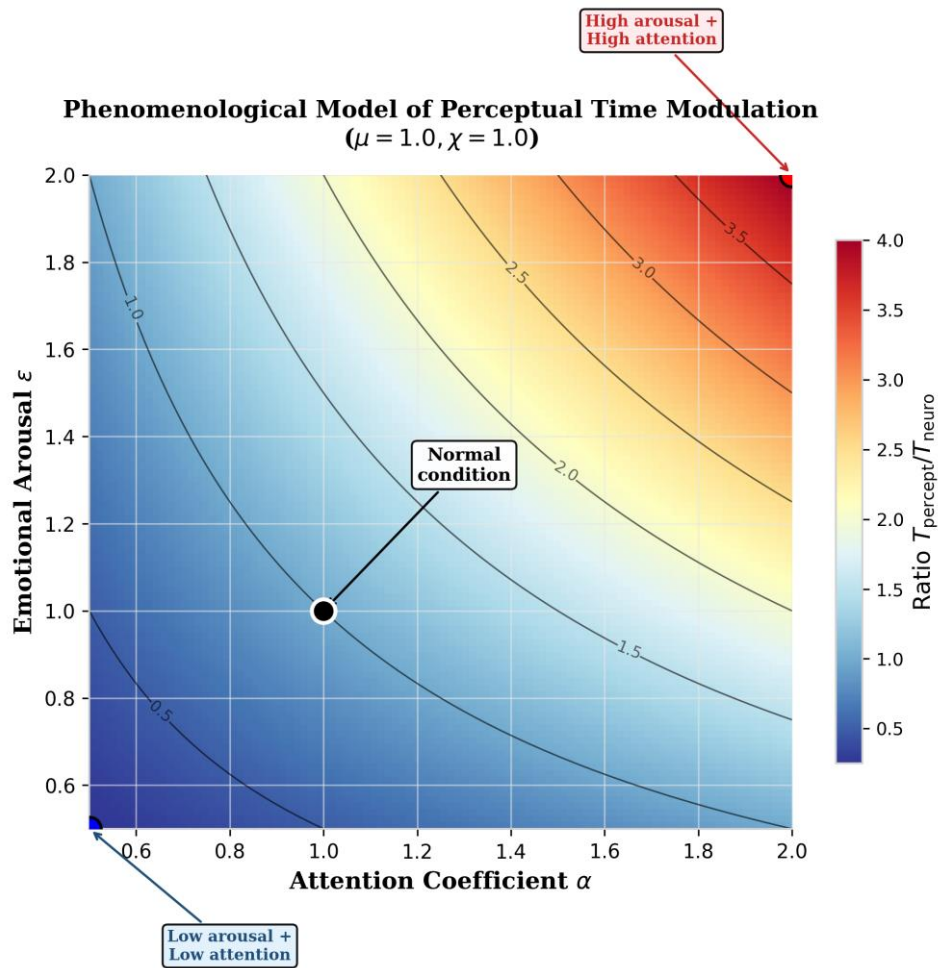


Figure 2. Phenomenological model of perceptual time modulation. The heatmap shows the ratio $T_{\text{percept}} / T_{\text{neuro}}$ as a function of the attention coefficient α and affective excitement ϵ , with $\mu = \chi = 1.0$. Contour lines indicate constant ratio values. The black ring marks the normal condition ($\alpha = \epsilon = 1.0$, ratio = 1.0). The red circle shows high arousal and high attention ($\alpha = \epsilon = 2.0$, ratio = 4.0), corresponding to subjective time growth. The blue circle indicates low excitement and low focus ($\alpha = \epsilon = 0.5$, ratio = 0.25), corresponding to subjective time compression.

We directly note that Eq. 11 is a phenomenological model intended to illustrate conceptual relationships, not a quantitatively predictive equation. The coefficients lack unique operational definitions and would require extensive empirical calibration for specific applications.

3. The Integrated Biophysical Model and Transit Scenarios

3.1. Hierarchical Relationships and Constraints

The four temporal layers form a strict hierarchy with constraints:

$$T_{\text{coord}} \xrightarrow{\text{Lorentz}} T_{\text{proper}} \xrightarrow{\text{Biology}} T_{\text{neuro}} \xrightarrow{\text{Cognition}} T_{\text{percept}} \quad (12)$$

with the critical constraint $T_{\text{neuro}} \leq T_{\text{proper}}$. Physical time provides the spacetime framework, neural processing operates within that framework, and perceptual time emerges from neural activity.

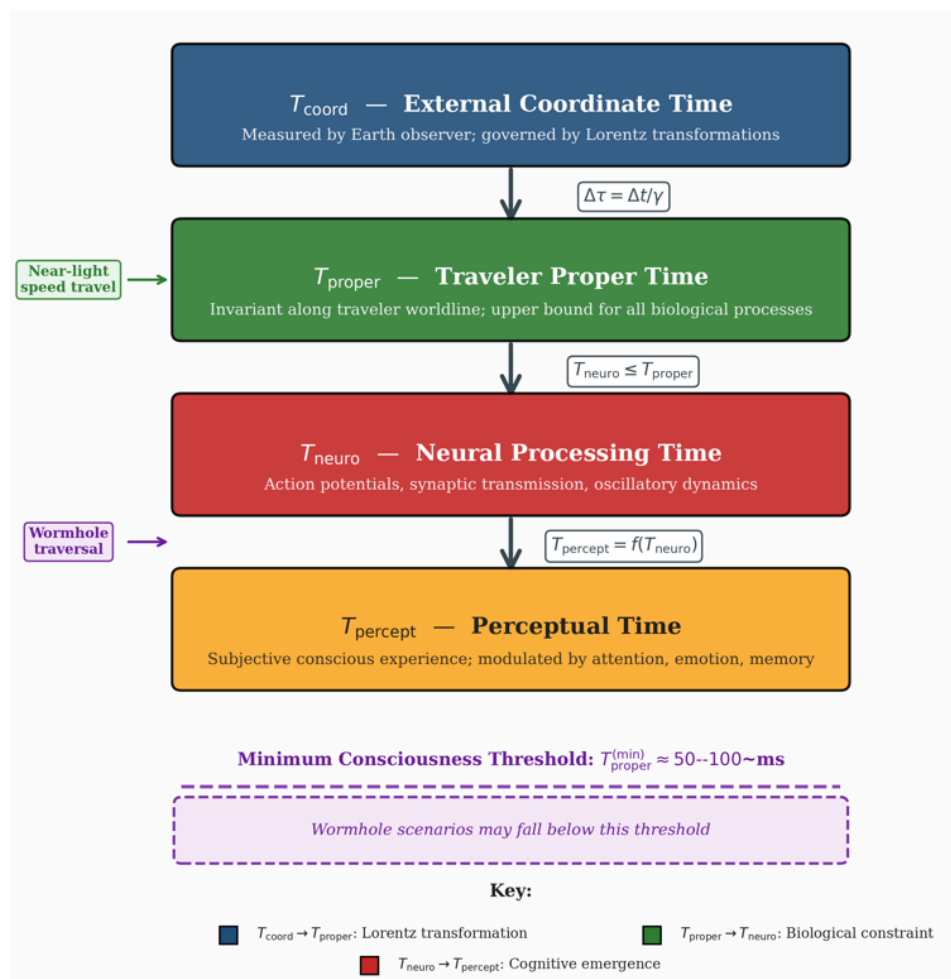


Figure 3. Layered model of temporal layers. External align time (T_{coord}) is transformed by unique relativity into the traveller's appropriate time (T_{proper}). Neural processing time (T_{neuro}) is constrained to not exceed T_{proper} . Perceptual time (T_{percept}) emerges from T_{neuro} through mental modulation. The minimum boundary for awareness ($\sim 50 - 100$ ms) is indicated. Wormhole scenarios may fall below this threshold.

3. 2. Case Study: Near-Light-Speed Interstellar Transit

Consider a journey of $D = 10$ light-years at $v = 0.999999c$:

Coordinate time (Earth observer):

$$T_{\text{coord}} = \frac{D}{v} \approx \frac{10 \text{ ly}}{0.999999c} \approx 10 \text{ years} \quad (13)$$

Proper time (traveler):

$$T_{\text{proper}} = \frac{T_{\text{coord}}}{\gamma} = \frac{10 \text{ years}}{707.1} \approx 12.4 \text{ hours} \quad (14)$$

Neural processing capacity: With $T_{\text{proper}} \approx 12.4$ hours = 44,640 s, and a gamma cycle period $T_\gamma = 1/40 \text{ Hz} = 25 \text{ ms}$:

$$N_\gamma = \frac{T_{\text{proper}}}{T_\gamma} = \frac{44,640 \text{ s}}{0.025 \text{ s}} \approx 1.79 \times 10^6 \quad (15)$$

The traveller has approximately 1.8 million gamma cycles available. Given the brain's parallel architecture ($\sim 10^{11}$ neurones, $\sim 10^{15}$ synapses), this provides substantial computational capacity for rich conscious experience. The constraint $T_{\text{neuro}} \leq T_{\text{proper}}$ is easily satisfied. The dramatic difference between coordinate time and proper time, and the resulting neural processing capacity, is visualised in [Figure 4](#).

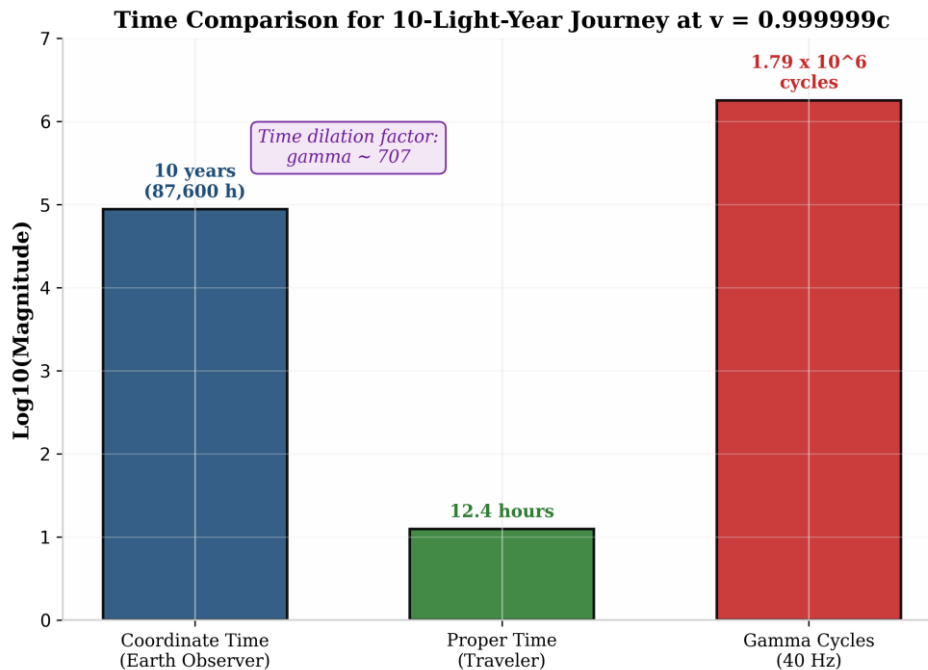


Figure 3. Contrast of temporal scales for a 10-light-year journey at $v = 0.999999c$. The coordinate time seasoned by the Earth observer (10 years) is compressed by a factor of $\gamma \approx 707$ to 12.4 hours of proper time for the traveller. Within this proper time, the brain can complete approximately 1.79×10^6 gamma cycles (40 Hz), providing substantial computational capacity for conscious knowledge.

Memory formation: Working memory and sensory integration operate on millisecond-to-second timescales and can occur during transit. Long-term memory integration (requiring seconds to hours via synaptic potentiation and hippocampal-cortical dialogue) may be partially limited. The traveler may retain vivid impressions rather than detailed episodic memories.

3.3. Case Study: Wormhole Traversal as Boundary Condition

For a hypothetical wormhole with throat length $l = 100$ m traversed at $v = 0.99c$:

$$T_{\text{proper}} = \frac{100 \text{ m}}{0.99 \times 3 \times 10^8 \frac{\text{m}}{\text{s}}} \approx 3.37 \times 10^{-7} \text{ s} \approx 337 \text{ ns} \quad (16)$$

This is $\sim 7.4 \times 10^4$ times shorter than the ~ 25 ms gamma period. Therefore:

$$T_{\text{proper}} \ll T_{\text{proper}}^{(\min)} \rightarrow \text{No sustained conscious experience} \quad (17)$$

The traversal would be experienced as effectively instantaneous. For conscious experience to be possible during wormhole transit, the geometry would need to provide considerably greater proper time (e.g., throat length $\gtrsim 30,000$ km at $0.99c$ yielding $\gtrsim 0.1$ s).

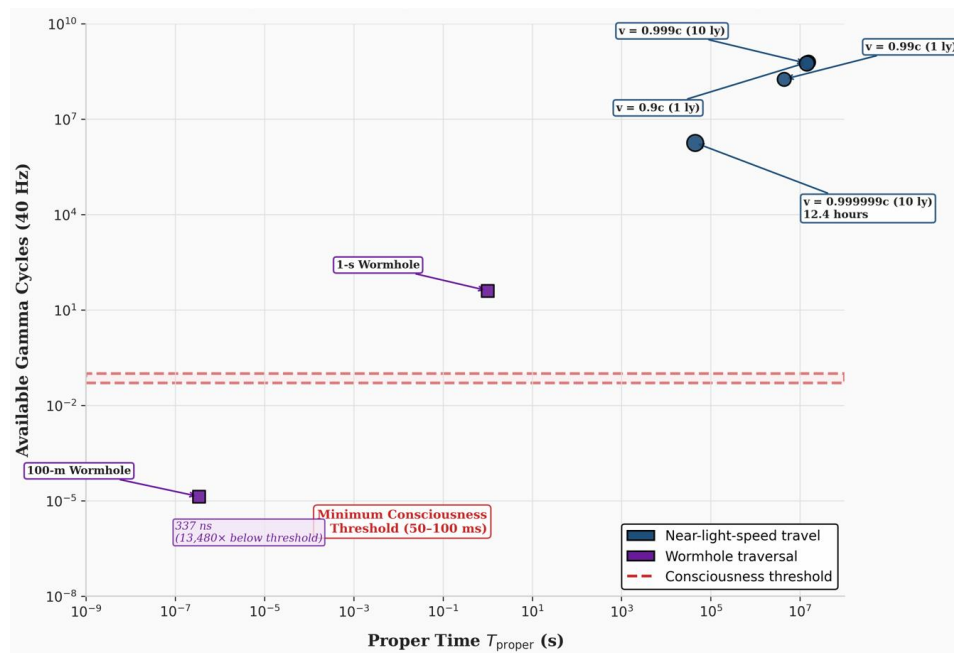


Figure 4. Relationship between proper time and available gamma cycles (40 Hz) across transit scenarios. The red dashed line indicates the minimum threshold for conscious experience ($\sim 50 - 100$ ms, $2 - 4$ gamma cycles). The 100-m wormhole scenario falls far below the threshold, while near-light-speed travel provides ample proper time.

3.4. Testable Predictions and Validation Protocols

We distinguish between near-term and long-term testable predictions:

3. 4. 1. Near-Term Predictions (Virtual Reality and Patient Studies)

P1 (VR Paradigm): Virtual reality experiments simulating time dilation effects should demonstrate that participants' neural processing times (measured via EEG/MEG) remain unchanged in their local reference frame, while perceptual time estimates shift. Validation: Compare EEG gamma power and reaction times against subjective duration reports under simulated dilation.

P2 (Patient Studies): Patients with temporal processing disorders should show specific patterns of neural oscillation disruption corresponding to the $T_{\text{neuro}} \rightarrow T_{\text{percept}}$ transformation. Validation: Correlate gamma/theta band abnormalities with duration estimation tasks in ADHD, depression, and schizophrenia cohorts.

3. 4. 2. Long-Term Predictions (Spaceflight and Computational Models)

P3 (Spaceflight): During high-speed transit (even at sub-relativistic speeds), astronauts should show normal neural processing times as measured by EEG but altered circadian rhythms due to changed external time cues. Validation: Longitudinal EEG and cognitive assessment during deep-space missions.

P4 (Computational): Biologically realistic neural network simulations under simulated relativistic conditions should confirm that information processing time remains constant in the network's reference frame. Validation: Implement spiking neural networks with appropriate time constants and measure internal processing vs. external time dilation.

4. Discussion

4. 1. Implications for Neuroscience

4. 1. 1. A Taxonomy for Temporal Processing Disorders

Our framework suggests a taxonomy for disorders involving temporal processing:

- ❖ **Type 1 (T_{neuro} dysfunction):** Parkinson's disease, ADHD—pathological changes in pacemaker-accumulator mechanisms.
- ❖ **Type 2 ($T_{\text{neuro}} \rightarrow T_{\text{percept}}$ decoupling):** Depression, schizophrenia—intact neural timing but distorted subjective experience via attentional/emotional modulation.
- ❖ **Type 3 ($T_{\text{proper}} \rightarrow T_{\text{neuro}}$ constraint violation):** Hypothetical relativistic scenarios where proper time is insufficient for required neural processing.

This classification may guide targeted interventions: dopaminergic agents for Type 1, cognitive training for Type 2, and, speculatively, neurotechnological interfaces for Type 3.

4. 1. 2. The Temporal Binding Problem

Our model raises questions about how the brain integrates information across temporal scales. We propose that temporal binding operates at the $T_{\text{neuro}} \rightarrow T_{\text{percept}}$ interface through nested oscillatory windows (Varela, 1999): gamma (25 – 40 ms) for perceptual moments, theta (125 – 250 ms) for working memory updates, and delta (250 – 2000 ms) for conscious integration.

4. 2. Implications for Space Travel and Biomedical Engineering

4. 2. 1. Consciousness Monitoring and Preservation

Our framework identifies specific biomedical engineering challenges for interstellar travel:

- ❖ **Challenge 1:** Temporal Disorientation. Astronauts experiencing extreme time dilation face a disconnect between T_{percept} and $T_{\text{coord,Earth}}$. Engineering response: Develop neurotechnological interfaces (augmented reality displays, direct neural feedback) providing real-time synchronisation cues.
- ❖ **Challenge 2:** Neural Integrity. Extreme acceleration could compromise neural function. Engineering response: Neuroprotective agents, gel-based suspension systems for mechanical stabilisation, and haemodynamic monitoring maintaining constant cerebral perfusion.
- ❖ **Challenge 3:** Consciousness Verification. During transit, standard behavioural assessments may be impossible. Engineering response: Implantable or wearable neural interfaces for real-time monitoring of gamma oscillations and evoked potentials to verify sustained T_{neuro} within T_{proper} constraints.

4. 2. 2. Proposed Biomedical Engineering Protocols

We propose the following engineering framework for relativistic spaceflight:

- ❖ **Pre-flight baseline:** Establish individual neural timing parameters, including gamma frequency, conduction velocities, and synaptic delays, via high-density EEG and transcranial magnetic stimulation.
- ❖ **In-flight monitoring:** Continuous EEG/ECOG monitoring of gamma-band activity (30–100 Hz) to verify $T_{\text{neuro}} \leq T_{\text{proper}}$ and detect consciousness degradation.
- ❖ **Adaptive life support:** Closed-loop systems adjusting neuroprotective agent delivery based on real-time neural signal analysis.

Post-flight assessment: Comprehensive neurocognitive evaluation comparing pre- and post-flight temporal processing capabilities.

4. 3. Limitations and Future Directions

4. 3. 1. Theoretical Limitations

- ❖ **L1:** This framework is conceptual and lacks direct experimental validation. No human has experienced extreme relativistic conditions.
- ❖ **L2:** Our neural model simplifies massively parallel, hierarchical processing. Future work should incorporate multi-scale dynamical systems approaches ([Breakspear, 2017](#)).
- ❖ **L3:** We have not integrated quantum gravitational effects, which may become relevant at Planck-scale regimes.
- ❖ **L4:** The mechanism by which neural activity generates consciousness (the “hard problem”) remains unspecified. We assume consciousness requires neural activity without asserting a specific mechanism.

4. 3. 2. Future Research

- **Computational neuroscience models:** Biologically realistic spiking neural networks under simulated relativistic time dilation to test prediction P4.
- **VR paradigms:** Immersive environments manipulating apparent time flow while recording EEG/fMRI to test P1.
- **Spaceflight analogs:** Studies in isolation chambers, microgravity (ISS), and centrifuge environments to test temporal perception under altered physical conditions.
- **Mathematical formalization:** Rigorous category-theoretic or fiber bundle formulations of the temporal layer coupling.
- **Integration with predictive coding:** Connections to active inference and Bayesian brain hypotheses about time perception ([Friston, 2010](#)).

5. Conclusion

The presented conceptual framework distinguishes four temporal layers coordinate time, proper time, neural processing time, and perceptual time to analyse the conditions necessary for conscious experience during extreme relativistic transit. Our analysis yields four principal conclusions:

First, special relativity does not create a paradox for conscious experience in the traveller's frame; rather, it establishes strict constraints. All neural processes are bounded by the traveller's proper time ($T_{\text{neuro}} \leq T_{\text{proper}}$). There is no mechanism by which biological processing can exceed this invariant limit.

Second, we derive a minimum proper time threshold of approximately 50–100 ms (2–4 gamma cycles) for sustained conscious perception. Near-light-speed travel over interstellar distances provides ample proper time (e.g., 12.4 hours for a 10-light-year journey at $0.999999c$), supporting rich subjective experience. Conversely, certain hypothetical spacetime shortcuts may fall below this threshold, predicting absent or degraded consciousness during transit.

Third, neuropsychiatric disorders provide terrestrial analogues for temporal layer decoupling, particularly at the $T_{\text{neuro}} \rightarrow T_{\text{percept}}$ interface. These analogues illustrate mechanisms of temporal distortion without implying that psychiatric conditions involve relativistic physics.

Fourth, we propose specific biomedical engineering protocols for consciousness monitoring during future spaceflight, including neural interface design, real-time gamma-band monitoring, and adaptive neuroprotection systems. This framework is explicitly hypothetical and is intended to stimulate interdisciplinary research at the physics-neuroscience interface. It generates testable predictions through computational modelling, virtual reality paradigms, and patient studies, while acknowledging that direct validation of relativistic consciousness will require advances in propulsion technology beyond current capabilities.

Acknowledgments

The authors thank colleagues at the College of Medical Technology, Benghazi, for discussions on interdisciplinary research methodology. This work received no specific grant from any funding agency.

Conflict of Interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Data Availability

No empirical data were generated for this conceptual study. All mathematical calculations and theoretical derivations are presented in the manuscript.

References

- Barkley, R. A., Koplowitz, S., Anderson, T., & McMURRAY, M. B. (1997). Sense of time in children with ADHD: Effects of duration, distraction, and stimulant medication. *Journal of the International Neuropsychological Society*, 3(4), 359–369. <https://doi.org/10.1017/S1355617797003597>
- Block, R. A., Hancock, P. A., & Zakay, D. (2010). How cognitive load affects duration judgments: A meta-analytic review. *Acta Psychologica*, 134(3), 330–343. <https://doi.org/10.1016/j.actpsy.2010.03.006>
- Breakspear, M. (2017). Dynamic models of large-scale brain activity. *Nature Neuroscience*, 20(3), 340–352. <https://doi.org/10.1038/nn.4497>
- Buhusi, C. V., & Meck, W. H. (2005). What makes us tick? Functional and neural mechanisms of interval timing. *Nature Reviews Neuroscience*, 6(10), 755–765. <https://doi.org/10.1038/nrn1764>
- Buzsáki, G., & Draguhn, A. (2004). Neuronal Oscillations in Cortical Networks. *Science*, 304(5679), 1926–1929. <https://doi.org/10.1126/science.1099745>
- Carroll, S. (2004). *Spacetime and Geometry: An Introduction to General Relativity* (Addison-Wesley, San Francisco) p 1.
- Clément, G., & Reschke, M. F. (2008). *Neuroscience in Space*. Springer New York. <https://doi.org/10.1007/978-0-387-78950-7>
- Coull, J. T., Vidal, F., Nazarian, B., & Macar, F. (2004). Functional Anatomy of the Attentional Modulation of Time Estimation. *Science*, 303(5663), 1506–1508. <https://doi.org/10.1126/science.1091573>
- Droit-Volet, S., & Meck, W. H. (2007). How emotions colour our perception of time. *Trends in Cognitive Sciences*, 11(12), 504–513. <https://doi.org/10.1016/j.tics.2007.09.008>
- Eagleman, D. M. (2008). Human time perception and its illusions. *Current Opinion in Neurobiology*, 18(2), 131–136. <https://doi.org/10.1016/j.conb.2008.06.002>
- Einstein, A. (1905). On the electrodynamics of moving bodies. In *The Roots of Special Relativity* (pp. 1a–23). Routledge. <https://www.taylorfrancis.com/chapters/edit/10.4324/9781315023687-2/electrodynamics-moving-bodies-einstein>
- Einstein, A. (1916). The General Theory of Relativity. In A. Einstein, *The Meaning of Relativity* (pp. 54–75). Springer Netherlands. https://doi.org/10.1007/978-94-011-6022-3_3
- Friston, K. (2010). The free-energy principle: A unified brain theory? *Nature Reviews Neuroscience*, 11(2), 127–138. <https://doi.org/10.1038/nrn2787>
- Gibbon, J., Church, R. M., & Meck, W. H. (1984). Scalar timing in memory. *Annals of the New York Academy of Sciences*, 423(1), 52–77.
- Ivry, R. B., & Schlerf, J. E. (2008). Dedicated and intrinsic models of time perception. *Trends in Cognitive Sciences*, 12(7), 273–280. <https://doi.org/10.1016/j.tics.2008.04.002>
- Kitamura, T., & Kumar, R. (1982). Time passes slowly for patients with depressive state. *Acta Psychiatrica Scandinavica*, 65(6), 415–420. <https://doi.org/10.1111/j.1600-0447.1982.tb00865.x>
- Malapani, C., Rakitin, B., Levy, R., Meck, W. H., Deweer, B., Dubois, B., & Gibbon, J. (1998). Coupled Temporal Memories in Parkinson's Disease: A Dopamine-Related Dysfunction. *Journal of Cognitive Neuroscience*, 10(3), 316–331. <https://doi.org/10.1162/089892998562762>
- Manzey, D., & Lorenz, B. (1998). Mental performance during short-term and long-term spaceflight. *Brain Research Reviews*, 28(1–2), 215–221. [https://doi.org/10.1016/S0165-0173\(98\)00041-1](https://doi.org/10.1016/S0165-0173(98)00041-1)
- Matthews, W. J., & Meck, W. H. (2016). Temporal cognition: Connecting subjective time to perception, attention, and memory. *Psychological Bulletin*, 142(8), 865–907. <https://doi.org/10.1037/bul0000045>
- Merchant, H., Harrington, D. L., & Meck, W. H. (2013). Neural Basis of the Perception and Estimation of Time. *Annual Review of Neuroscience*, 36(1), 313–336. <https://doi.org/10.1146/annurev-neuro-062012-170349>
- Misner, C. W., Thorne, K. S., & Wheeler, J. A. (1973). *Gravitation* WH Freeman and Co. San Francisco, 660.

- Morris, M. S., & Thorne, K. S. (1988). Wormholes in spacetime and their use for interstellar travel: A tool for teaching general relativity. *American Journal of Physics*, 56(5), 395–412. <https://doi.org/10.1119/1.15620>
- Newton, I. (1687). *Philosophiæ naturalis principia mathematica* (Mathematical principles of natural philosophy). London (1687), 1687(1687), 1687.
- Ornstein, R. E. (1975). On the experience of time. <https://psycnet.apa.org/record/1976-08735-000>
- Pöppel, E. (1997). A hierarchical model of temporal perception. *Trends in Cognitive Sciences*, 1(2), 56–61. [https://doi.org/10.1016/S1364-6613\(97\)01008-5](https://doi.org/10.1016/S1364-6613(97)01008-5)
- Pöppel, E. (2009). Pre-semantically defined temporal windows for cognitive processing. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364(1525), 1887–1896. <https://doi.org/10.1098/rstb.2009.0015>
- Purves, D., Augustine, G. J., Fitzpatrick, D., Katz, L. C., LaMantia, A.-S., McNamara, J. O., & Williams, S. M. (2001). Neurotransmission in the visceral motor system. In *Neuroscience*. 2nd edition. Sinauer Associates. <https://www.ncbi.nlm.nih.gov/books/NBK10854/>
- Tse, P. U., Intriligator, J., Rivest, J., & Cavanagh, P. (2004). Attention and the subjective expansion of time. *Perception & Psychophysics*, 66(7), 1171–1189. <https://doi.org/10.3758/BF03196844>
- Van Ombergen, A., Demertzi, A., Tomilovskaya, E., Jeurissen, B., Sijbers, J., Kozlovskaya, I. B., Parizel, P. M., Van De Heyning, P. H., Sunaert, S., Laureys, S., & Wuyts, F. L. (2017). The effect of spaceflight and microgravity on the human brain. *Journal of Neurology*, 264(S1), 18–22. <https://doi.org/10.1007/s00415-017-8427-x>
- Varela, F. J. (1999). The specious present: A neurophenomenology of time consciousness. <https://psycnet.apa.org/record/2000-07254-008>
- Volz, H.-P., Nenadic, I., Gaser, C., Rammsayer, T., Häger, F., & Sauer, H. (2001). Time estimation in schizophrenia: An fMRI study at adjusted levels of difficulty. *Neuroreport*, 12(2), 313–316.
- Wiener, M., Turkeltaub, P., & Coslett, H. B. (2010). The image of time: A voxel-wise meta-analysis. *NeuroImage*, 49(2), 1728–1740. <https://doi.org/10.1016/j.neuroimage.2009.09.064>
- Wittmann, M. (2013). The inner sense of time: How the brain creates a representation of duration. *Nature Reviews Neuroscience*, 14(3), 217–223. <https://doi.org/10.1038/nrn3452>
- Zivi, P., Sdoia, S., Ventura, R., Ferravante, D., & Ferlazzo, F. (2026). Challenges for cognitive research in spaceflight and analog environments. *iScience*, 29(5), 115790. <https://doi.org/10.1016/j.isci.2026.115790>