

JOURNAL OF MEDICINE AND HEALTH SCIENCES



VOLUME 33 • NUMBER 2 • AUGUST 2026

■ CLINICAL AND BASIC
MEDICAL SCIENCES

Advancing knowledge,
improving patient care

■ NURSING, ALLIED HEALTH SCIENCE
AND PUBLIC HEALTH

Promoting health, preventing disease,
strengthening communities

■ MEDICAL AND HEALTH SCIENCES
EDUCATION

Inspiring minds, shaping the future
of health care

Editorial Board

Advisors

- **Assistant Professor Nantana Choomchuay, M.D.**
Dean, Faculty of Medicine, Srinakharinwirot University
- **Associate Professor Dr. Malai Taweechoitpatr, Ph.D.**
Associate Dean for Research, Faculty of Medicine, Srinakharinwirot University

Editor-in-Chief

- **Associate Professor Dr. Chatchai Ekpanyaskul, M.D., SFHEA**
Department of Preventive and Social Medicine, Faculty of Medicine, Srinakharinwirot University

Assistant Editor

- **Assistant Professor Dr. Ratchadaporn Pramong, Ph.D.**
Department of Anatomy, Faculty of Medicine, Srinakharinwirot University
- **Lecturer Dr. Nanticha Fahromkao, M.D., Ph.D.**
Department of Preventive and Social Medicine, Faculty of Medicine, Srinakharinwirot University

Editorial Board Members

- **Emeritus Professor Dr. Songsak Petmitr, Ph.D.**
Department of Molecular Tropical Medicine and Genetic, Faculty of Tropical Medicine, Mahidol University
- **Emeritus Professor Dr. Kosum Chansiri, Ph.D.**
Srinakharinwirot University
- **Professor Dr. Narin Hiransuthikul, M.D., Ph.D.**
Department of Preventive and Social Medicine, Faculty of Medicine, Chulalongkorn University
- **Professor Dr. Sarawut Jitrapakdee, Ph.D.**
Department of Biochemistry, Faculty of Science, Mahidol University
- **Professor Dr. Ammarin Thakkinstan, Ph.D.**
Department of Clinical Epidemiology and Biostatistics, Faculty of Medicine Ramathibodi Hospital, Mahidol University
- **Associate Professor Suthat Rungruanghiranya, M.D.**
Department of Medicine, Faculty of Medicine, Kasetsart University
- **Associate Professor Thawatchai Tullavardmana, M.D.**
Department of Surgery, Faculty of Medicine, Srinakharinwirot University
- **Associate Professor Nuntigar Sonsuwan, M.D.**
Department of Otolaryngology, Faculty of Medicine, Chiang Mai University
- **Associate Professor Dr. Nuanchan Chutabhakdikul, Ph.D.**
Research Center for Neuroscience, Institute of Molecular Biosciences, Mahidol University
- **Associate Professor Dr. Narumon Leelayuwat, Ph.D.**
Department of Physiology, Faculty of Medicine, Khon Kaen University
- **Assistant Professor Sathit Kurathong, M.D.**
Department of Medicine Vajira Hospital, Navamindradhiraj University

Managing Editor

- **Miss Phueksa Chaemsai**
Research Division, Faculty of Medicine, Srinakharinwirot University
- **Miss Tiraporn Tukaew**
Research Division, Faculty of Medicine, Srinakharinwirot University

Editorial Note

A New Policy on the Use of Generative Artificial Intelligence and AI-Assisted Technologies

The Journal of Medicine and Health Sciences is a peer-reviewed academic journal published by the Faculty of Medicine, Srinakharinwirot University, Thailand. Since its establishment in 1994, the Journal has served as an academic platform for disseminating scholarly work in medicine, health sciences, public health, and related disciplines. Over the past three decades, the Journal has remained committed to advancing scientific knowledge, promoting interdisciplinary research, and supporting the translation of evidence into clinical practice, public health policy, and community well-being.

In line with the mission of Srinakharinwirot University and the Faculty of Medicine to generate knowledge for the benefit of society, the Journal particularly values research that addresses complex health challenges through multidisciplinary perspectives. Contemporary health issues increasingly require collaboration among basic biomedical sciences, clinical medicine, epidemiology, public health, allied health sciences, health technology, and medical or health science education. Reflecting this broad academic scope, the present issue includes diverse contributions in biomedical research, clinical research, public health, allied health, and health innovation.

All submitted manuscripts are evaluated through a rigorous double-blind peer-review process by three independent experts in relevant fields. The Journal is also firmly committed to maintaining high standards of research integrity and publication ethics. Authors are expected to comply with internationally recognized ethical principles and editorial standards, including those recommended by the International Committee of Medical Journal Editors (ICMJE) and the Committee on Publication Ethics (COPE). These standards are essential for ensuring responsible authorship, transparency, appropriate disclosure of conflicts of interest, accuracy of reporting, and reproducibility of scientific findings.

The rapid development of generative artificial intelligence and AI-assisted technologies has created new opportunities as well as new challenges for scholarly publishing. These tools may assist authors in improving language, organizing information, or enhancing readability. However, inappropriate or undisclosed use of AI may compromise scientific integrity, particularly when it involves generated figures, fabricated or inaccurate references, unverified content, or lack of transparency regarding how AI tools were used. Such practices also increase the workload

of editors, reviewers, and the editorial office in verifying the originality, accuracy, and reliability of submitted manuscripts.

At the same time, the Journal recognizes the transformative potential of artificial intelligence (AI) in advancing medicine, health sciences, and scholarly communication. AI technologies are increasingly contributing to scientific discovery, clinical decision-making, health education, data analysis, and research productivity. The Journal therefore supports the responsible and ethical use of AI and AI-assisted technologies when they enhance scientific quality, promote innovation, and ultimately contribute to improvements in healthcare and societal well-being. Nevertheless, technological advancement must be accompanied by appropriate governance, transparency, and human oversight to ensure that scientific integrity and public trust in research are preserved.

Recognizing these concerns, the Journal of Medicine and Health Sciences has introduced a new policy and guideline on the use of generative artificial intelligence and AI-assisted technologies. This policy applies not only to authors, but also to reviewers and members of the editorial team. Its purpose is to promote transparency, human accountability, confidentiality, proper disclosure, and research integrity throughout the publication process. Authors who use AI-assisted tools in the preparation of manuscripts are required to disclose such use clearly and to take full responsibility for the accuracy, originality, and integrity of the final submitted work. Reviewers and editors must also ensure that confidential manuscript materials are handled responsibly and are not inappropriately shared with AI systems.

The full policy and author guidelines are available on the Journal website. The editor-in-chief encourages all contributors, reviewers, and readers to become familiar with these requirements. By implementing this policy, the Journal of Medicine and Health Sciences reaffirms its commitment to international editorial standards and to the responsible advancement of scientific knowledge. The Journal embraces innovation and supports the appropriate use of artificial intelligence to accelerate progress in medicine and health sciences, while maintaining the highest standards of research integrity, transparency, and ethical scholarship. Through this balanced approach, the Journal will continue to promote high-quality research that advances evidence-based medicine, improves health outcomes, and serves society.

Associate Professor Dr. Chatchai Ekpanyaskul, M.D., SFHEA
Editor-in-Chief

Table of Contents

Original Article

Comparing Prognostic Potential of Ki-67 in Breast Cancer Between Hotspot and Global Scoring by Digital Image Analysis	76
<i>Panut Achintharangkoon Supparat Changmuenwai Pubet Janyakulwong Potsawat Kosonvittayatham Sirikorn Chusri Varintorn Charoenchol</i>	
Association Between Postpartum Duration and Work Ability Among Working-Age Postpartum Women: An Analytical Cross-Sectional Study	81
<i>Krittin Nakaphadungrat Naesinee Chaiear Phanumas Krisorn</i>	
Preventing Post-Inflammatory Hyperpigmentation After Laser Treatment of Solar Lentigines: A Randomized Controlled Trial of Corticosteroids and Brimonidine	91
<i>Pakagamon Tumsutti Salinee Rojhirunsakool Nanticha Faromkao Saranya Khunkhet Nathathai Prathumchat Montree Udompataikul</i>	
Protective Effect of Mixed Probiotic Culture Against <i>Salmonella</i> Typhimurium Infection in Mice	101
<i>Jirakit Supamanta Chattramat Jaiyim Kittiya Khongkool Chantanapa Chantarangkul Praewpannarai Jamjuree Kaikwa Wuttisan Anongnard Kasorn Malai Taweechotipatr</i>	
Comparative Analysis of Asthma Control Assessment Questionnaires in Children Using the Global Initiative for Asthma (GINA) Guidelines: A Prospective Cohort Study	112
<i>Prapassinee Suntipraron</i>	
Efficacy of Using Interactive Video Learning on Medical Students' Satisfaction, Knowledge, and Engagement in a Large-Group Family Medicine Orientation Session	119
<i>Passorn Rotpenpian</i>	
Outcome of Nursing Service Process Improvement on Waiting Time for Coronary Angiography in Patients with Non-ST-Elevation Myocardial Infarction (NSTEMI)	127
<i>Crisaporn Poonsong</i>	
Diagnostic Utility of Bile Cytology for Cholangiocarcinoma: A Real-World Comparison of ERCP-Guided Brush Cytology and PTBD-Derived Bile Cytology	134
<i>Panunat Muangnoi Panitpong Maroongroge Chanchai Charonpongsuntorn Supagone Wangsubtawee Asawin Sudcharoen Passagorn Sangsawangchot</i>	
Predictive Value of AIMS65 Score for Clinical Outcomes in Thai Cirrhotic Patients with Upper Gastrointestinal Bleeding	143
<i>Natthawat Aranyawat</i>	
Impact of the COVID-19 Pandemic on STEMI Care Processes and Treatment Outcomes in the Emergency Department of a University Hospital	150
<i>Worapot Khampeera Parinya Tianwibool</i>	
Analysis of Patients Coded for Cutaneous Nontuberculous Mycobacterial Infections (ICD-10 A31.1) Using Thai Inpatient Data from 2020 to 2024	159
<i>Lapasrada Pattarapreeyakul Sunisa Kanchanasutthiyakorn Pichanee Watthanasiri Rujiraporn Pitaksalee Kan Khemnakh Sarrote Boonkerd Pramet Kaewmesri Monpat Chamnanphon Surakameth Mahasirimongkol</i>	
Trend Analysis and Factors Associated with the Results of Rifampicin-Resistant Tuberculosis Testing Using the Gene Xpert MTB/RIF in Patients at Pathum Thani Hospital	169
<i>Putthapach Chailuang</i>	

ORIGINAL ARTICLE

Comparing Prognostic Potential of Ki-67 in Breast Cancer Between Hotspot and Global Scoring by Digital Image Analysis

Panut Achinthanangkoon¹, Supparat Changmuenwai², Pubet Janyakulwong², Potsawat Kosonvittayatham², Sirikorn Chusri², Varintorn Charoenchol²

¹Department of Pathology, Faculty of Medicine, Srinakharinwirot University

²Faculty of Medicine, Srinakharinwirot University

KEYWORDS

breast cancer; Ki-67; hotspot scoring; global scoring; digital image analysis

CORRESPONDING AUTHOR

Panut Achinthanangkoon

Department of Pathology, Faculty of Medicine, Srinakharinwirot University
62 Village No.7, Rangsit-Nakhon Nayok Road, Ongkharak Sub-district, Ongkharak District, Nakhon Nayok 26120, Thailand
E-mail: panut@g.swu.ac.th

Received: December 23, 2025

Revised: April 18, 2026

Accepted: April 22, 2026

ABSTRACT

Breast cancer remains one of the leading causes of cancer-related mortality in women worldwide. The Ki-67 proliferation index is widely used as a biomarker to assess tumor aggressiveness; however, significant variability persists due to subjective visual interpretation and the lack of consensus on whether hotspot or whole-slide global scoring provides superior prognostic value. With the integration of digital slide scanning and image analysis technology at the HRH Princess Maha Chakri Sirindhorn Medical Center, opportunities have emerged to enhance scoring accuracy and reduce observer-related variability. This retrospective cohort study aimed to compare the prognostic potential of Ki-67 hotspot versus global scoring using digital image analysis in breast cancer patients. A total of 71 patients aged over 18 years who underwent surgery and pathological evaluation between 2019 and 2020 were included. Survival outcomes were analyzed using Kaplan–Meier and Cox regression models. For this analysis, Ki-67 proliferation indices were dichotomized using a 20% threshold (low <20% vs. high ≥20%). The results demonstrated no statistically significant association between recurrence-free survival and Ki-67 assessed by either hotspot scoring ($p=0.4395$) or global scoring ($p=0.6805$). Overall survival analysis could not be reliably performed due to the limited number of death events. Several factors may have contributed to the absence of significant findings, including the relatively small sample size, the younger mean age of the cohort, and potential bias introduced during manual hotspot selection. These findings highlight the need for future studies with larger populations, automated hotspot detection algorithms, and extended follow-up durations to more accurately determine the prognostic utility of Ki-67 quantification methods in breast cancer. Strengthening methodological rigor and standardization will be essential in guiding the clinical application of Ki-67 in prognostic stratification and treatment decision-making.

Introduction

Breast cancer is currently one of the most common malignancies in women. Each cancer subtype differs in aggressiveness, growth rate, and overall prognosis. In current clinical practice, patients diagnosed with breast cancer typically undergo Ki-67 immunohistochemical (IHC) staining on formalin-fixed paraffin-embedded (FFPE) tissue. Ki-67 antigen is a nuclear protein expressed during the G1, S, G2, and M phases of the cell cycle, but absent in resting (G0) cells. Therefore, an elevated Ki-67 index correlates with increased cellular proliferation and poorer prognosis, and is reported as the percentage of tumor cells showing positive staining¹. Ki-67 is widely accepted and commonly used for assessing tumor aggressiveness in various malignancies, including breast, lung, prostate, cervical, and central nervous system cancers²⁻⁴.

For breast cancer, the International Ki-67 in Breast Cancer Working Group (IKWG) has reported that, among patients with T1-T2, N0-N1, estrogen receptor-positive and HER2-negative tumors, Ki-67 ≤ 5% is associated with a favorable prognosis, whereas Ki-67 ≥ 30% indicates a significantly poorer outcome⁵. IKWG recommends counting 500–1,000 tumor cells under a ×40 objective lens, but no consensus has been established regarding whether counting should be performed in the area of highest proliferation (hotspot) or as an overall average (global)⁶. In routine practice, Ki-67 scoring is visually assessed by pathologists, but the results may vary due to interobserver subjectivity.

A previous comparison study reported that hotspot counting of 500 tumor cells yielded a lower interclass correlation coefficient (ICC) than global counting, where four areas of 100 tumor cells each were evaluated⁷. That

study included 23 pathologists from 12 countries. With the advancement of digital pathology, digital image analysis (DIA) has gained increasing attention as a tool for objective quantification. Zhong et al.⁸ demonstrated that ICC values for visual Ki-67 assessment were lower than those obtained using DIA for both hotspot and global counting. Nevertheless, visual counting remains widely used due to its convenience and speed.

The HRH Princess Maha Chakri Sirindhorn Medical Center has implemented a slide scanner system (3D-Histech, P-480), which enables digital whole-slide imaging and automated nuclear quantification. This technology offers potential advantages for more accurate Ki-67 assessment. Therefore, the present study aims to compare Ki-67 hotspot and global scoring using both visual assessment and digital image analysis and to correlate these scoring methods with clinical outcomes. The findings may help improve diagnostic reporting and breast cancer management within our institution.

Materials and Methods

Study Design

This retrospective cohort study was designed to assess the prognostic potential of Ki-67 hotspot versus whole-slide average (global) digital image analysis scoring in patients diagnosed with breast cancer who received treatment at HRH Princess Maha Chakri Sirindhorn Medical Center between January 1, 2019, and December 31, 2020.

Study Population

Inclusion Criteria

- Patients diagnosed with breast cancer and treated at the HRH Princess Maha Chakri Sirindhorn Medical Center between January 1, 2019, and December 31, 2020.
- Patients who underwent surgical excision and pathological examination at the Department of Pathology.
- Availability of immunohistochemical staining results for ER, PR, HER2, and Ki-67.
- Age > 18 years.

Exclusion Criteria

- Absence of Ki-67-stained slides or slides with staining too faint to be evaluated.

Data Collection Procedures

Clinical data of eligible breast cancer patients who underwent surgery at the HRH Princess Maha Chakri Sirindhorn Medical Center during the study period were retrieved from the electronic medical record (EMR) system. Patients were selected according to the predefined inclusion and exclusion criteria. Clinical variables collected included age, sex, tumor type, tumor size, number of metastatic lymph nodes, and results of immunohistochemical markers, obtained from medical records and pathology reports. All data were recorded in Microsoft Excel. For each case, Ki-67 immunostained slides were identified and scanned using a slide scanner. Digital image analysis software was used to quantify the Ki-67 proliferation index across the whole slide to obtain the global score. Subsequently, the region with the highest proliferative activity was selected, and the same software was used to determine the hotspot score.

In this study, “global scoring” refers to the Ki-67 proliferation index quantified across the entire annotated tumor area on the whole-slide image, excluding non-tumor components such as stroma, necrosis, lymphocytes, and normal ductal structures. Tumor regions were manually annotated by a pathologist using digital image analysis software, ensuring that only morphologically confirmed invasive carcinoma cells were included in the quantification. For “hotspot scoring,” the region with the highest density of Ki-67, positive tumor nuclei were visually identified by the pathologist within the annotated tumor area. A fixed field of view at $\times 40$ magnification (0.2 mm^2) was then analyzed using the same software. All measurements were recorded for further analysis using Stata version 14.0.

Statistical Analysis

Sample size estimation was performed using Stata version 14.0 to achieve 80% statistical power at a 5% significance level, with a maximum acceptable precision error of 5% and a 95% confidence interval. Based on a previous study by Stephanie Robertson et al., breast cancer patients with a hotspot Ki-67 score demonstrated a disease-free survival rate of 90%, while those with a Ki-67 DIA global score ($\geq 20\%$ vs $< 20\%$) exhibited a hazard ratio (HR) of 3.53. The required sample size was calculated to be 30 patients. Therefore, a minimum of 30 cases was deemed sufficient to address the primary objectives of this study.

Results

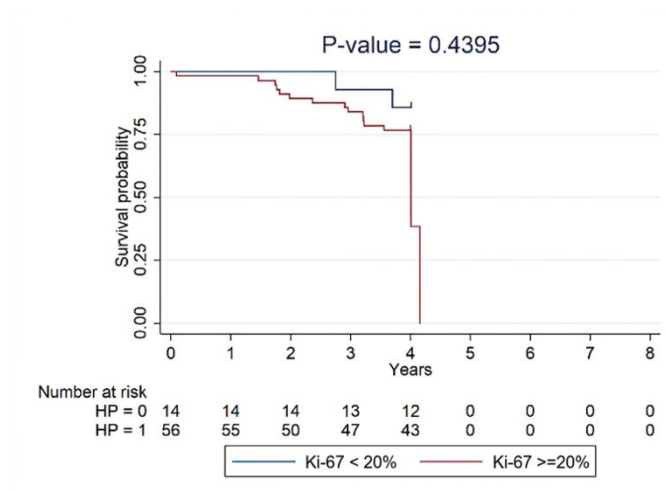
A total of 83 Ki-67–stained slides from patients diagnosed with breast cancer and treated at the HRH Princess Maha Chakri Sirindhorn Medical Center were initially reviewed. Twelve cases were excluded based on the predefined criteria: missing date of diagnosis ($n = 6$), missing date of recurrence ($n = 1$), and diagnosis dates outside the eligibility period ($n = 5$). Seventy-one cases were eligible for analysis, in which both global and hotspot Ki-67 scores were successfully quantified using the digital image analysis software. The mean age of the patients was 52.93 years, and all were female. The follow-up period was five years. Most tumors were classified as invasive breast carcinoma of no special type (ductal) ($n = 60$). The majority of cases were histologic grade 2 ($n = 36$). Lymph node status was predominantly negative ($n = 38$). Tumor size was most commonly between 2 and 5 cm ($n = 42$). Ki-67 proliferation indices were categorized into $< 20\%$ (low; $n = 14$) and $\geq 20\%$ (high; $n = 57$). A summary of baseline clinicopathologic characteristics is presented in Table 1.

Prognostic Potential of Ki-67 for Recurrence-Free Survival (RFS)

Kaplan–Meier survival analysis demonstrated no statistically significant association between recurrence-free survival (RFS) and the Ki-67 index measured using the hotspot scoring method ($p = 0.4395$), as illustrated in Figure 1. Similarly, no significant association was observed between RFS and Ki-67 global scoring obtained through digital image analysis ($p = 0.6805$), as shown in Figure 2. Analysis of overall survival (OS) was limited by the small number of events. Only three patients died during the 5-year follow-up period, all of whom died within the first year. Due to the insufficient number of deaths, meaningful statistical evaluation of the association between Ki-67 indices and OS could not be performed.

Table 1 Baseline clinicopathologic characteristics and survival outcomes of the study population

Variables	No.	%
Total no. of tumors	71	100
Patients mean age at diagnosis	52.93	-
Location		
Left	30	42.25
Right	41	57.25
Histological subtype		
Invasive breast carcinoma (ductal)	60	84.51
Invasive lobular carcinoma	2	2.82
Metaplastic carcinoma	1	1.41
Mucinous carcinoma	3	2.82
Invasive papillary carcinoma	2	1.41
Micropapillary CA	1	1.41
Invasive micropapillary carcinoma	1	1.41
Adenoid cystic carcinoma	1	1.41
Nottingham histological grade		
Not evaluation	1	1.41
Grade 1	5	7.04
Grade 2	36	50.70
Grade 3	29	40.85
Tumor size		
≤ 2 cm	22	30.99
> 2 and ≤ 5 cm	42	59.19
> 5 cm	7	9.86
No. of positive lymph node		
Positive	33	46.48
Negative	38	53.52
Skin or nipple involved		
Positive	2	2.82
Negative	69	97.18

**Figure 1** Kaplan–Meier curve showing the relationship between recurrence-free survival (RFS) and Ki-67 hotspot scoring.

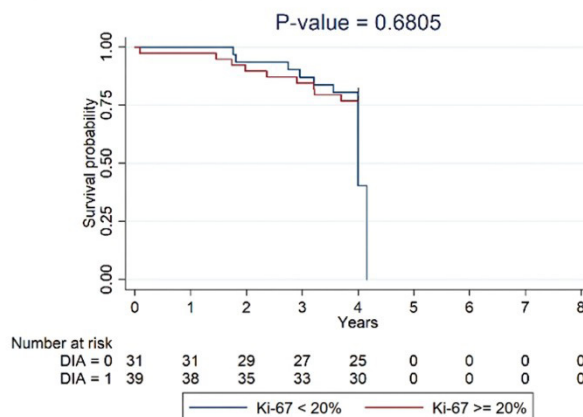


Figure 2 Kaplan–Meier curve demonstrating the relationship between recurrence-free survival (RFS) and the Ki-67 index measured using the global scoring method.

Prognostic Potential of Ki-67 Between DIA Global and DIA Hotspot Scoring

Further analysis using univariate Cox regression was performed to evaluate the prognostic potential of Ki-67 for recurrence-free survival (RFS), adjusting for histological grade as a confounding factor. The results showed no significant association between RFS and Ki-67 quantified by either global scoring (HR 1.24, 95% CI 0.44-3.49, $p = 0.681$) or hotspot scoring (HR 1.78, 95% CI 0.40-7.91, $p = 0.446$) using digital image analysis (Table 2).

Kaplan-Meier analyses (Figures 1-2) and Cox regression (Table 2) both evaluated Ki-67 using a consistent 20% cut-off. The distinction between methods lies in quantification strategy: hotspot scoring assessed the region of highest Ki-67 density at $\times 40$ magnification, whereas global scoring quantified the entire annotated tumor area. Both approaches employed identical digital image analysis algorithms. Neither method achieved statistical significance for recurrence-free survival with hotspot scoring showing a higher but less precise risk estimate (wider 95% CI), likely reflecting observer-dependent variability in manual region selection.

Table 2 Univariate Cox regression hazard models for recurrence-free survival

Variables	Hazard ratio	95% Confidence interval	p-value
Ki-67 DIA global scoring	1.24	0.44 - 3.49	0.681
Ki-67 DIA Hotspot scoring	1.78	0.40 - 7.91	0.446

Discussion

This study investigated the prognostic potential of the Ki-67 proliferative index in breast cancer using digital image analysis (DIA), comparing hotspot scoring with global scoring. The findings demonstrated that hotspot scoring showed a slightly better trend toward predicting recurrence-free survival (RFS) compared with global scoring; however, neither method reached statistical significance. This observation contrasts with the findings of Robertson et al., who reported that global scoring provides superior prognostic performance.

Several limitations may explain the discrepancies between the present study and previous reports, as well as the lack of statistical significance observed in our results. The mean age of patients in this study was 52.93 years, compared with 59 years in the study by Robertson. Age is an established prognostic factor in breast cancer, with patients over 60 years generally exhibiting poorer outcomes. Differences in age distribution may therefore have influenced the prognostic performance of Ki-67 across studies. The relatively small

sample size likely contributed to wide 95% confidence intervals and increased p -values, reducing the precision and statistical power of the analysis. Additionally, only three deaths occurred in this cohort, all within the first year of follow-up, making meaningful overall survival (OS) analysis impossible. These limitations are inherent to the retrospective cohort design and the available patient pool. Another important factor relates to hotspot selection. Although cell counting was performed using dedicated software, the identification of the hotspot region, the area with the highest Ki-67 expression, was conducted manually. This approach introduces potential instrumentation and observer bias. Furthermore, limitations of the software itself occasionally resulted in misclassification, such as failure to detect lightly stained tumor cells or inadvertent detection of non-tumor cells (e.g., stromal cells, lymphocytes). These issues may have reduced the reliability of Ki-67 quantification.

Although no statistically significant prognostic difference was observed between Ki-67 hotspot and global scoring using digital image analysis (DIA), our findings offer

practical insights for clinical implementation. First, manual hotspot selection remains susceptible to observer bias, suggesting that DIA should initially serve as an adjunct tool, paired with pathologist verification and internal validation, rather than a standalone diagnostic method. Second, Ki-67 values derived from semi-automated workflows should be interpreted within a comprehensive clinicopathological context and not used in isolation to guide adjuvant therapy decisions.

Future research should consider a larger sample size to improve statistical precision and narrow confidence intervals, thereby enhancing the reliability of prognostic estimates. Increasing the number of long-term follow-up events, particularly deaths, would allow for more robust analysis of overall survival. Age should also be considered more thoroughly, as the younger average age in the present cohort compared with previous studies may have influenced the prognostic value of Ki-67. Subgroup analyses stratified by age, particularly for patients over 60 years, may reveal more distinct prognostic patterns. Improvements in hotspot selection methodology are also recommended. Automated hotspot detection using advanced digital image analysis software may reduce observer-dependent bias and improve reproducibility. Long-term, prospective follow-up studies with standardized digital image analysis protocols may provide more definitive insights into the prognostic significance of Ki-67 in breast cancer.

Conclusions

Among breast cancer patients treated at the HRH Princess Maha Chakri Sirindhorn Medical Center between January 1, 2019, and December 31, 2020, Ki-67 indices assessed by either hotspot scoring or global scoring using digital image analysis showed no statistically significant association with recurrence-free survival or overall survival. Therefore, this study cannot conclusively determine the comparative prognostic performance of hotspot versus global Ki-67 scoring in this population.

Ethical statement

The study was approved by the Institutional Human Research Ethics Committee of Srinakharinwirot University (protocol code SWUEC-663201; approval date: March 5, 2024). Informed consent was obtained from all participants or their legal representatives.

References

- Li LT, Jiang G, Chen Q, et al. Ki67 is a promising molecular target in the diagnosis of cancer (review). *Mol Med Rep* 2015;11(3):1566-72. doi:10.3892/mmr.2014.2914.
- Ishihara M, Mukai H, Nagai S, et al. Retrospective analysis of risk factors for central nervous system metastases in operable breast cancer: Effects of biologic subtype and Ki67 overexpression on survival. *Oncology* 2013;84(3):135-40. doi:10.1159/000345321.
- Ciancio N, Galasso MG, Campisi R, et al. Prognostic value of p53 and Ki67 expression in fiberoptic bronchial biopsies of patients with non small cell lung cancer. *Multidiscip Respir Med* 2012;7(1):29. doi:10.1186/2049-6958-7-29.
- Josefsson A, Wikström P, Egevad L, et al. Low endoglin vascular density and Ki67 index in Gleason score 6 tumours may identify prostate cancer patients suitable for surveillance. *Scand J Urol Nephrol* 2012;46(4):247-57. doi:10.3109/00365599.2012.669791.
- Nielsen TO, Leung SCY, Rimm DL, et al. Assessment of Ki67 in breast cancer: Updated recommendations from the international Ki67 in breast cancer working group. *J Natl Cancer Inst* 2021;113(7):808-19. doi:10.1093/jnci/djaa201.
- Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: Recommendations from the international Ki67 in breast cancer working group. *J Natl Cancer Inst* 2011;103(22):1656-64. doi:10.1093/jnci/djr393.
- Leung SCY, Nielsen TO, Zabaglo LA, et al. Analytical validation of a standardised scoring protocol for Ki67 immunohistochemistry on breast cancer excision whole sections: An international multicentre collaboration. *Histopathology* 2019;75(2):225-35. doi:10.1111/his.13880.
- Zhong F, Bi R, Yu B, et al. A comparison of visual assessment and automated digital image analysis of Ki67 labeling index in breast cancer. *PLoS One* 2016;11(2):e0150505. doi:10.1371/journal.pone.0150505.
- Chen HL, Zhou MQ, Tian W, et al. Effect of age on breast cancer patient prognosis: A population-based study using the SEER 18 database. *PLoS One* 2016;11(10):e0165409. doi:10.1371/journal.pone.0165409.
- Stalhammar G, Robertson S, Wedlund L, et al. Digital image analysis of Ki67 in hot spots is superior to both manual Ki67 and mitotic counts in breast cancer. *Histopathology* 2018;72(6):974-89. doi:10.1111/his.13452.
- Koopman T, Buikema HJ, Hollema H, et al. Digital image analysis of Ki67 proliferation index in breast cancer using virtual dual staining on whole tissue sections: Clinical validation and inter-platform agreement. *Breast Cancer Res Treat* 2018;169(1):33-42. doi:10.1007/s10549-018-4669-2.
- Rewcastle E, Skaland I, Gudlaugsson E, et al. The Ki67 dilemma: Investigating prognostic cut-offs and reproducibility for automated Ki67 scoring in breast cancer. *Breast Cancer Res Treat* 2024;207(1):1-12. doi:10.1007/s10549-024-07352-4.
- Wu C-H, Chang M-H, Tsai H-H, et al. Assessment and visualization of Ki67 heterogeneity in breast cancers through digital image analysis. *Adv Breast Cancer Res* 2024;13(2):11-26. doi:10.4236/abcr.2024.132002.
- Zwager MC, Yu S, Buikema HJ, et al. Advancing Ki67 hotspot detection in breast cancer: A comparative analysis of automated digital image analysis algorithms. *Histopathology* 2025;86(2):204-13. doi:10.1111/his.15294.
- Skjervold AH, Pettersen HS, Valla M, et al. Visual and digital assessment of Ki-67 in breast cancer tissue - a comparison of methods. *Diagn Pathol* 2022;17(1):45. doi:10.1186/s13000-022-01225-4.
- Lashen A, Toss MS, Green AR, et al. Ki67 assessment in invasive luminal breast cancer: A comparative study between different scoring methods. *Histopathology* 2022;81(6):786-98. doi:10.1111/his.14781.

ORIGINAL ARTICLE

Association Between Postpartum Duration and Work Ability Among Working-Age Postpartum Women: An Analytical Cross-Sectional Study

Krittin Nakaphadungrat¹, Naesinee Chaiear², Phanumas Krisorn²

¹Master of Science Program in Occupational Medicine and Occupational Health (International Program), Faculty of Medicine, Khon Kaen University

²Department of Community, Family and Occupational Medicine, Faculty of Medicine, Khon Kaen University

KEYWORDS

work ability; postpartum women; return to work; occupational health

CORRESPONDING AUTHOR

Naesinee Chaiear

Department of Community, Family and Occupational Medicine, Faculty of Medicine, Khon Kaen University

123 Mittraphap Road, Nai Muang Sub-district, Muang District, Khon Kaen 40002, Thailand

E-mail: naesinee@kku.ac.th

Received: March 27, 2026

Revised: April 25, 2026

Accepted: April 28, 2026

ABSTRACT

This study aimed to compare the Work Ability Index (WAI) of postpartum women engaged in income-generating work before and after 6 weeks postpartum. This is a cross-sectional analytical study. The sample comprised postpartum mothers who attended Srinagarind Hospital between June and October 2025. The study divided participants into two groups: mothers who had worked before 6 weeks postpartum (99 cases) and those who had worked at 6 weeks postpartum or later (96 cases). The instruments included the Work Ability Index (WAI) and questionnaires on personal information, employment type, and job characteristics. Data were analyzed using descriptive statistics (percentages, means, and standard deviations) and inferential statistics (crude OR and AdjORs with 95% confidence intervals). The results showed that postpartum mothers who worked before 6 weeks were more likely to have less than a bachelor's degree, earn less than 15,000 baht, receive no compensation during maternity leave, and have no workplace welfare, compared to those who worked at 6 weeks or more. The WAI of postpartum mothers who worked before 6 weeks was 30.51 ± 4.43 , lower than the mean WAI of 35.30 ± 3.44 for those who worked at 6 weeks or later, and this difference was statistically significant ($p < 0.001$). Factors associated with poor WAI among postpartum mothers included working before 6 weeks (AdjOR 3.27, 95% CI: 1.26-8.51), being an informal worker (AdjOR 5.67, 95% CI: 1.98-16.40), and being aged 30 years or younger (AdjOR 2.95, 95% CI: 1.15-7.55). When analyzing informal and formal workers separately, and across age groups ≤ 30 years and > 30 years, working before 6 weeks postpartum was associated with a poorer WAI. In conclusion, postpartum mothers who worked before 6 weeks had a significantly lower mean WAI than those who worked at 6 weeks or later ($p < 0.001$) and working before 6 weeks postpartum was associated with poorer work ability, highlighting the need for improved maternity protection and workplace support.

Introduction

Maternal leave is an important issue for postpartum women and the newborn. Postpartum women should receive at least 18 weeks of maternity leave according to WHO¹, or at least 98 days according to the Thai Labor Protection Act², and ILO³. Notably, the Thai Labor Protection Act² increased this duration to 120 days in December 2025, just two months after the last participant was recruited. According to the American Medical Association (AMA), the optimal recovery duration for mothers with physical disabilities due to childbirth, including the recovery period, is at least 42 days, regardless of the mode of childbirth or the intensity of work⁴. These recommendations or regulations can only be applied to the workers in the formal labor system (both government sections and private company sections) but not to informal workers² (defined as workers not covered by labor or government regulations because they operate

outside the formal system; this includes local business owners, the self-employed, employees of unregistered companies, or those in non-compliant hiring arrangements, such as workers for whom employers fail to remit mandatory Social Security contributions).

The return-to-work situation was examined in a study⁵ on the proportion of women by duration after childbirth, compared with developed countries, including Thailand⁵, the United States⁶, and France⁷. Thirty-six percent of Thai mothers returned to work within 1 month after childbirth⁵, whereas in the United States and France, 7% and $< 5\%$, respectively, returned to work within 1 month. The proportion of early return to work in Thailand is much higher because of Thailand's informal labor structure, which had approximately 25.1 million informal workers (64.3% of all workers in 2013)⁸ and 21 million informal workers (52.3% of all workers in 2023)⁹. Informal workers are typically

not covered by labor protection laws, including maternity leave benefits. As a result, many postpartum women in this group may be compelled to return to work earlier than medically recommended. This structural context provides a key rationale for the present study, which examines the impact of early return-to-work on work ability among postpartum women.

The report showed that approximately one-third of all postpartum women in Thailand worked 4 weeks after childbirth, even though the American Medical Association (AMA) considers this period to be the period of disability or recovery⁴. However, it remains unclear whether postpartum women can safely return to work with full job demands during the early postpartum period.

Currently, numerous tools exist to evaluate the functional capacity of postpartum women across various domains, including physical and mental ability, as well as the impact of health status on work performance. However, it is impractical to utilize every available instrument within a single study. A widely recognized tool in the research community for exploring these comprehensive aspects is the Work Ability Index (WAI)¹⁰, developed by Professor Ilmarinen. The WAI is a self-report questionnaire covering seven primary dimensions: physical demands, mental demands, work performance, diagnosed diseases, sick

leave, the impact of health on work, and mental resources. Because the WAI can effectively distinguish between different groups—including postpartum groups before and after the six-week physiological healing period—it was chosen for this study. Figure 1 illustrated conceptual framework which included three domains: (1) Occupational factors, measured by employment type (formal vs. informal), job characteristics (e.g., blue-collar vs. white-collar work), working hours, and occupational hazards. These variables represent job demands and exposure that directly influence work ability. (2) Individual factors, measured by age, education level, maternal health conditions (e.g., wound pain, sleep deprivation, mental health status), and number of comorbidities. These factors reflect the physical and psychological capacity of postpartum women. (3) Organizational/environmental factors, measured by access to compensation, workplace welfare, and income stability. These variables represent external support systems that may facilitate or hinder recovery and work ability. These domains were hypothesized to influence Work Ability Index (WAI) both directly and indirectly.

This study aimed (1) to compare WAI in postpartum women before and after 6 weeks postpartum and (2) to identify factors associated with poor work ability during this period.

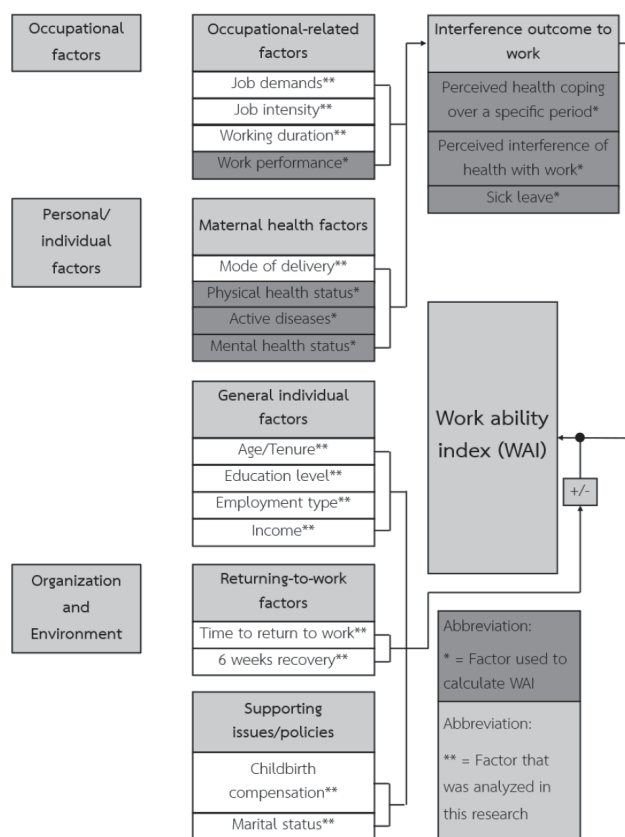


Figure 1 Conceptual framework of factors associated with poor WAI.

Materials and Methods

Study population

This cross-sectional analytical study explored WAI among postpartum women who performed income-generating tasks by comparing two groups: those working within 6 weeks after childbirth and those working after 6 weeks. The target population comprised postpartum women aged 20–35 years who were within 10 weeks postpartum, visited Srinagarind Hospital between June and October 2025, and subsequently returned to work. The study population comprised individuals who met the inclusion criteria: postpartum women aged 20–35 years (all delivery modes) who worked for income at 4–6 weeks or after 6 weeks postpartum (8–12 weeks), were able to communicate in Thai, and provided informed consent—with justification for the age range based on shared metabolic and physiological characteristics commonly grouped in clinical studies. Exclusion criteria were mothers with irreversible disability within six months postpartum due to pregnancy or other causes preventing return to work, as well as non-Thai speakers.

Sample size determination

To determine the means of each group of working mothers, the statistical formula to calculate sample size was $N = (Z * \sigma / E)^2$, in which N is the sample size, Z is the Z -score for the 95% confidence interval, σ is the standard deviation (SD) of the target population, and E is the margin of error (MOE). For this study, the MOE was set at 20% of the SD. This value was chosen because the research focuses on social and behavioral sciences and serves as a preliminary study regarding postpartum mothers. Setting the MOE at 20% of the SD ensures an error margin of no more than 0.5 from the mean. Furthermore, this guarantees that the error remains below 10% of the mean Work Ability Index (WAI) found in general research, where 10% of the mean WAI typically centers around 3. The sample size was confirmed using WinPEPI version 11.65 for estimating the means. The mothers in the working group < 6 weeks (which had the simulated SD of 2.20) had a 20% MOE of 0.44 and a required sample size of 99, and the mothers in the working group $\geq$ 6 weeks (which had the simulated SD of 1.33) had a 20% MOE of 0.27 and a required sample size of 96. Therefore, 195 participants were deemed sufficient to achieve the desired level of precision across both groups.

Sample selection

Convenience sampling was employed; eligible participants who visited the hospital represented random variation; all who met the inclusion criteria were recruited until the required sample size was achieved. In the clinical context, this approach was appropriate because mothers routinely attend scheduled visits at specific time points after childbirth, enabling systematic recruitment during regular working hours at two hospital clinics: the Pediatric Vaccination Clinic, where mothers brought their infants for routine vaccinations at 8–12 weeks postpartum, and the Postpartum Clinic, where mothers attended for standard body checks at 6 weeks postpartum. However, it is acknowledged that convenience sampling may introduce selection bias, as mothers with certain personal or extreme health-related issues might miss their scheduled appointments or fail to visit the physician entirely, thus leading to their underrepresentation in the study.

Tools development

The questionnaire comprised two components: (1) demographic, occupational, and maternal health information, and (2) the WAI based on Ilmarinen's principle¹⁰ in Finnish¹¹, which was translated into Thai¹². Items in Part 1 were developed from relevant literature and the clinical context. The overall principle behind each question was followed. Three experts specialized in (1) obstetrics, (2) occupational medicine, and (3) questionnaire development. Following revision, all items achieved an Item-Content Validity Index (I-CVI) of 1.00. To ensure reliability, a pretest was conducted with 10 postpartum women to assess clarity and congruence. The responses for each question aligned with the expected outcomes, demonstrating the reliability and stability of the instrument. Furthermore, the WAI is a well-established tool within the Thai research community; repeated testing across various regions and populations in Thailand¹² (also included young women) consistently yields predictable reliability coefficients (Cronbach's alpha) above 0.70. Finally, minor wording adjustments were made based on feedback from the pretest group before the main data collection. For example, misspellings were corrected; the BMI item was removed because it was difficult for the general population to calculate and was replaced with weight and height; and the sex item (male/female) was excluded because the study population consisted solely of women.

Data collection

Recruitment was conducted at the Postpartum and Pediatric Vaccination Clinics within Srinagarind Hospital, Faculty of Medicine, Khon Kaen University. Trained members of the research team or nurses screened all postpartum women attending the clinics according to the inclusion and exclusion criteria. Eligible participants were selected using convenience sampling. Once individual targets were identified, participants were invited to complete a self-administered questionnaire after receiving study information and providing written informed consent. Data collection continued until the predetermined sample size of 195 participants (< 6 weeks: 99 participants; $\geq$ 6 weeks: 96 participants) was reached. Data were collected between June and October 2025. All responses were checked for completeness and coded to maintain confidentiality before participants left the clinic. During the study period, 199 postpartum women were assessed for eligibility. Of these, 2 did not return the questionnaire, and 2 denied completing the critical parts when asked by the research team. A total of 195 participants were included in the final analysis (response rate: 98.0%).

Data management and analysis

Completed questionnaires were reviewed for accuracy by research assistants upon submission, who verified the correctness and completeness of responses and entered them into a secure database. WAI scores were interpreted according to Ilmarinen's criteria^{11–12}. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 28.0.1.0 (IBM Corp., Armonk, NY, USA.). A two-sided p -value < 0.05 was considered statistically significant in all analyses. Descriptive statistics, including means, standard deviations, frequencies, percentages, crude ORs, and AdjORs, were used to analyze demographic characteristics, return-to-work duration, and WAI scores. Inferential

analysis was conducted using binary logistic regression to examine the association between early return to work (< 6 weeks postpartum) and poor work ability (WAI ≤ 36). Both crude ORs and AdjOR with 95% confidence intervals (CI) were reported. A *p*-value < 0.05 was considered statistically significant. Poor or worse WAI was defined as WAI ≤ 36 based on Ilmarinen's classification, in which good or better WAI was > 36. This threshold has been widely used in occupational health research to distinguish suboptimal work ability from acceptable levels¹⁰. The postpartum working period was categorized as < 42 days or ≥ 42 days postpartum. This 42-day threshold was selected based on clinical evidence that the postpartum recovery period typically lasts 42 days, after which a mother's physiological state generally returns to baseline. In addition, the cutoff of 7-year experiences is the common period that workers gain enough experience to manage team as well as subordinates without supervision¹³. Furthermore, the WAI cutoff of 36 was utilized as it is the established standard for distinguishing "Poor" from "Good" work ability in the general population—a metric that has been extensively validated and deemed reliable. This study applies the WAI to postpartum mothers under the medical assumption that they have returned to a normal or near normal physiological state. In addition, the outcomes showed the odds of several factors in relation to poor WAI. These factors included age, education level, employment type, method of childbirth, average income, compensation, and gap time between childbirth and return to work. Associations with poor WAI were assessed using 95% confidence intervals, and *p*-values were reported to identify potential significant factors.

To control potential confounding, multivariable logistic regression analysis was conducted. Variables were selected based on both statistical criteria (*p* < 0.20 in univariate analysis) and theoretical relevance derived from the conceptual framework (e.g., age, education level, employment type, and postpartum working period). Multicollinearity between variables was assessed using correlation coefficients, and variables with high correlation ($|r| \geq 0.8$) were excluded to prevent instability in the model. AdjOR were then estimated to account for confounding effects and to identify independent associations with poor work ability. Model performance was assessed using the Hosmer–Lemeshow goodness-of-fit test to evaluate calibration, and the omnibus test of model coefficients to assess overall model significance.

Results

The sample consisted of postpartum mothers from June to October 2025 who attended Srinagarind Hospital. They were divided into two groups: postpartum mothers who worked before completing 6 weeks postpartum (99 cases) and those who worked at 6 weeks or more (96 cases). Most participants had poor WAI scores. In the < 6 weeks group, the mean WAI was 30.51 ± 4.43 , with 89.9% classified as poor. In the ≥ 6 weeks group, the mean was 35.30 ± 3.44 , with 68.8% classified as poor. The difference between the two groups was statistically significant (*p* < 0.001). Most participants in both groups were aged 20–30 years, accounting for 61.6% in the < 6 weeks group and 56.3% in the ≥ 6 weeks group. The most common mode of delivery in both groups was Caesarean section, reported in 55.6% of the < 6 weeks group and 52.1% of the ≥ 6 weeks group. Nearly all participants in both groups lived with their partners (97.0%).

Regarding educational attainment, most participants in the < 6 weeks group had less than a bachelor's degree (67.7%), whereas 51.0% in the ≥ 6 weeks group had a bachelor's degree or higher. The most frequently reported category for average monthly income was ≥ 15,001 baht, with 50.5% and 66.7% in the < 6 weeks and ≥ 6 weeks groups, respectively.

Employment benefits differed markedly between the two groups. Among women in the < 6 weeks group, the majority reported having no compensation (79.8%) and no workplace welfare (85.9%). In contrast, the ≥ 6 weeks received compensation (61.5%) and welfare (56.3%). This disparity reflects differences in employment types. Women returning later (> 6 weeks) were more likely to be engaged in formal employment systems, whereas those returning earlier (< 6 weeks) relied primarily on self-payment, reflecting informal work arrangements (Table 1).

The mean age of participants in the < 6 weeks group was 28.6 ± 4.2 years, compared with 29.48 ± 3.85 years in the ≥ 6 weeks group. The normality of WAI score distribution in both groups (before and after 6 weeks) was assessed using IBM SPSS Statistics for Windows, version 28.0.1.0 (142), and outliers were excluded. A paired-samples *t*-test was then conducted to examine changes in WAI scores over the 6-week period. The results revealed a significant decrease in WAI scores, with a mean difference of -4.79 (SD = 5.52), $t(95) = -8.50$, *p* < 0.001, indicating that participants' work ability significantly declined from before to after the 6-week period.

Table 1 Demographic characteristics of postpartum women working before and after 6 weeks postpartum

Variables	Number (%)	
	< 6 weeks n=99 (%)	≥ 6 weeks n=96 (%)
Age ≤ 30 years (n=115)	61 (61.6)	54 (56.3)
Mean age (SD) (yrs)	28.6 (4.2)	29.5 (3.9)
Min-Max of age (yrs)	20-35	21-35
Childbirth delivery as caesarean section (n=105)	55 (55.6)	50 (52.1)
Total number of childbirths ≥ 2 (n=83)	45 (45.5)	38 (39.6)
Living together with a couple (n=189)	96 (97.0)	93 (96.9)

Table 1 Continued

Variables	Number (%)	
	< 6 weeks n=99 (%)	≥ 6 weeks n=96 (%)
Highest education below bachelor's degree (n=116)	67 (67.7)	49 (51.0)
Average income per month (15,000 or less) (n=81)	49 (49.5)	32 (33.3)
No compensation during parental leave	79 (79.8)	37 (38.5)
No employment welfare (n=127)	85 (85.9)	42 (43.8)

Focusing on occupational characteristics by postpartum status, the < 6 weeks group was predominantly engaged in informal employment (79.8%), unstable income (76.8%), and manual or blue-collar work (53.5%), whereas the ≥ 6 weeks group mainly comprised formal workers (61.5%), those with stable income (58.3%), and office-based or white-collar occupations (67.7%). The mean work experience was comparable between the two groups, averaging 6.26 ± 2.67 years in the < 6 weeks group and 6.77 ± 3.01 years in the ≥ 6 weeks group. Occupational hazards that can cause direct damage to the body (non-psychological hazard) were reported more frequently among workers in the < 6 weeks group (60.6%), while psychological hazards were more common among workers in the ≥ 6 weeks group (56.3%).

Most participants in both groups reported jobs requiring combined physical and cognitive demands, accounting for more than 70% in each group. Most workers in both groups worked more than 8 h per day and more than 5 days per week, with higher proportions observed among workers in the < 6 weeks group. Regarding Ilmarinen's WAI components, *self-reported poor work performance* was markedly higher in the < 6 weeks group than in the ≥ 6 weeks group (90.9% vs. 26.0%, OR 28.40, 95%CI: 12.47-64.67, $p < 0.001$). However, the *self-reported good perception score regarding interference of health with work* was higher in the group < 6 weeks (62.6% vs 20.8%, OR 6.37, 95%CI: 3.33-12.50, $p < 0.001$). In addition, many variables reviewed the same pattern and this difference was statistically significant, including *being an informal worker* (OR 6.30, 95%CI: 3.32-11.95), *workplace provided not stable income* (OR 4.63, 95%CI: 2.50-8.33) (Table 2).

Table 2 Occupational characteristics by postpartum status

Factors	Number (%)	
	< 6 weeks n=99 (%)	≥ 6 weeks n=96 (%)
1. Being informal worker (n=116)	79 (79.8)	37 (38.5)
2. Workplace provided not stable income (n=116)	76 (76.8)	40 (41.7)
3. Work manners described by ISCO-08 were manual / labor tasks (Blue collar) (n=84)	53 (53.5)	31 (32.3)
4. Work experience < 7 years (n=90)	50 (50.5)	40 (41.7)
5. Main occupational hazards had direct damage to body (i.e. biological, biomechanics, chemical, physical, others) (n=102)	60 (60.6)	42 (43.8)
6. Works required both physical and cognitive (n=146)	76 (76.8)	70 (72.9)
7. Moderate job intensity (n=107)	60 (60.6)	47 (49.0)
8. Working more than 8 hours per day (n=149)	82 (82.8)	67 (69.8)
9. Working more than 5 days per week (n=163)	85 (85.9)	78 (81.3)
10. Self-reported poor work performance (Used in Ilmarinen's calculation) (n=115)	90 (90.9)	25 (26.0)
11. Self-reported good perception score toward health coping over a specific period (Used in Ilmarinen's calculation) (n=129)	71 (71.7)	58 (60.4)
12. Self-reported good perception score toward interference of health with work (Used in Ilmarinen's calculation) (n=82)	62 (62.6)	20 (20.8)

Regarding maternal factors, significant differences between the < 6 weeks and ≥ 6 weeks groups were observed for wound pain at vaginal or surgical sites, which was reported more often in the < 6 weeks group (34.3% vs. 11.5%, OR 4.05, 95%CI: 1.89-8.33, $p < 0.001$). Regarding Ilmarinen's factors, the following factors were more prevalent in the < 6 weeks group: *self-report poor mental health status* (79.8% vs. 51.0%, OR 2.01, 95%CI: 2.01-7.34, $p < 0.001$), *number*

of all active diseases more than 2 (71.7% vs. 53.1%, OR 2.24, 95%CI: 1.23-4.00, $p = 0.007$), and *poor attitude score toward 2 years expectations* (70.7% vs. 42.7%, OR 3.24, 95%CI 1.79, 5.86, $p < 0.001$) (Table 3). The *Good attitude toward 2-month expectation* refers to the participant's perceived ability to cope with current work and health conditions over the short term, whereas *2-year expectation* reflects long-term outlook and perceived sustainability in Ilmarinen's dimension.

Table 3 Maternal factors and returning to work results

Maternal factors	Number (%)	
	< 6 weeks n=99 (%)	≥ 6 weeks n=96 (%)
1. Without pregnancy related to underlying diseases (n=118)	64 (64.6)	54 (56.3)
2. Having wound pain (both vaginal /surgical site) (n=45)	34 (34.3)	11 (11.5)
3. Having mastitis/breast engorgement (n=35)	15 (15.2)	10 (10.4)
4. Having sleep deprivation (n=102)	60 (60.6)	42 (43.8)
5. Having postpartum blue/ depression (n=34)	22 (22.2)	12 (12.5)
6. Self-reported poor physical health status (Used in Ilmarinen's calculation) (n=165)	88 (88.9)	77 (80.2)
7. Self-reported poor mental health status (Used in Ilmarinen's calculation) (n=128)	79 (79.8)	49 (51.0)
8. Number of all active diseases more than 2 (Used in Ilmarinen's calculation) (n=122)	71 (71.7)	51 (53.1)
9. Length of hospital stay /sick leave in 1 year was 9 days or less (Used in Ilmarinen's calculation) (n=170)	89 (89.9)	81 (84.4)
10. Good attitude score toward 2 months expectation (n=129)	71 (71.7)	58 (60.4)
11. Poor attitude score toward 2 years expectation (n=111)	70 (70.7)	41 (42.7)

To control potential confounding, multivariable logistic regression analysis in SPSS version v28.0.1.0 (142) was performed to estimate AdjOR for factors associated with poor work ability (WAI ≤ 36). Variables were selected based on both statistical significance in multivariate model ($p < 0.05$) and theoretical relevance according to the conceptual framework. Collinearity between variables was assessed using correlation coefficients, and variables with high correlation ($|r| \geq 0.8$) were excluded from the final model to avoid multicollinearity.

The factors significantly associated with poor WAI (score ≤ 36) were those with education below a bachelor's degree, who were more likely to have poor WAI than those with a bachelor's degree or higher (OR = 3.83, 95% CI: 0.93-13.84), however, this factor was not significant. Participants assessed within 6 weeks postpartum had substantially higher odds of poor WAI than those assessed later (OR = 3.271, 95% CI: 1.26-8.51). Age ≤ 30 years also associated with poorer WAI (OR = 2.950, 95% CI: 1.15-7.55). Informal workers had markedly increased odds compared with formal workers (OR = 5.695, 95% CI: 1.98-16.4). Experiencing a direct impact from an occupational hazard was also associated with a lower WAI (OR = 2.313, 95% CI: 0.42-12.64) (Table 4).

Table 4 The analysis of factors associated with poor WAI

Factors	Number of poor WAI: n (%)	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p-value
1. Mother working at the period				
< 6 weeks (n=99)	89 (89.9)	4.045 (1.85, 8.85)	3.271 (1.26, 8.51)	0.015*
≥ 6 weeks (n=96)	66 (68.8)			
2. Age of mother				
≤ 30 years (n=115)	98 (85.2)	2.326 (1.15, 4.72)	2.950 (1.15, 7.55)	0.024*
> 30 years (n=80)	57 (71.2)			

Table 4 Continued

Factors	Number of poor WAI: n (%)	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	p-value
3. Childbirth method				
Caesarean section (n=105)	85 (81.0)	1.214 (0.61, 2.43)	0.933 (0.40, 2.18)	0.874
Others (n=90)	70 (77.8)			
4. Marital status				
Living together (n=189)	151 (79.9)	1.988 (0.35, 11.24)	0.65 (0.08, 5.58)	0.694
Others (n=6)	4 (66.7)			
5. Highest education				
Below bachelor's degree (n=116)	101 (87.1)	3.117 (1.52, 6.41)	3.583 (0.93, 13.84)	0.064
Bachelor's degree or above (n=79)	54 (68.4)			
6. Average income per month				
≤ B15,000 (n=81)	68 (84.0)	1.623 (0.78, 3.38)	0.553 (0.14, 2.16)	0.394
> B15,000 (n=114)	87 (76.3)			
7. Formality of work				
Informal work (n=116)	107 (92.2)	4.758 (2.24, 10.10)	5.695 (1.98, 16.40)	0.001*
Formal work (n=79)	48 (60.8)			
8. Worker classification				
Blue collar (n=84)	70 (83.3)	1.529 (0.74, 3.15)	0.836 (0.18, 3.80)	0.817
White collar (n=111)	85 (76.6)			
9. Current work experience				
≥ 7 years (n=105)	86 (81.9)	1.377 (0.69, 2.76)	0.454 (0.17, 1.19)	0.109
< 7 years (n=90)	69 (76.7)			
10. Main occupational hazard				
Physical hazard (n=102)	83 (81.4)	1.274 (0.63, 2.56)	2.313 (0.42, 12.64)	0.333
Other hazards (n=93)	72 (77.4)			
11. Demand of work				
Multiple demands work (n=146)	121 (82.9)	2.137 (1.01, 4.50)	0.702 (0.27, 1.81)	0.464
Single demand work (n=49)	34 (69.4)			
12. Job intensity				
Moderate or more (n=108)	87 (80.6)	1.157 (0.58, 2.33)	0.975 (0.35, 2.74)	0.961
Low (n=87)	68 (78.2)			
13. Working duration per day				
> 8 hrs. (n=149)	123 (82.6)	2.070 (0.97, 4.41)	1.044 (0.28, 3.86)	0.949
≤ 8 hrs. (n=46)	32 (69.6)			
14. Working duration per week				
> 5 days (n=163)	132 (81.0)	1.667 (0.70, 3.95)	1.345 (0.32, 5.70)	0.688
≤ 5 days (n=32)	23 (71.9)			

*Statistical significance

From the significant associations of factors and poor WAI in Table 5, the results showed that 3 factors were associated with poor WAI: working period (before or after 6 weeks), age (> 30 years), and employment status (informal or formal). Subgroup analyses were conducted by stratifying participants according to age group (≤ 30 vs. > 30 years) and employment type (formal vs. informal) to explore whether the association between return-to-work timing and work ability differed across key demographic and occupational

strata. These subgroup analyses were pre-specified based on the conceptual framework. The outcome showed that the worker aged ≤ 30 years had higher odds of poorer WAI when working before 6 weeks postpartum (OR 6.767, 95% CI: 1.83-25.09). However, the remaining subgroups (workers aged > 30 years, whether formal or informal) showed higher ORs for poorer WAI when working before 6 weeks postpartum, but the results were not significant (Table 5).

Table 5 Subgroup analysis of WAI between the working groups at “< 6 weeks” and “ ≥ 6 weeks” postpartum

Subgroup analysis of WAI between the working groups at “< 6 weeks” and “ ≥ 6 weeks” postpartum	Poor WAI n (%)	Crude Odds Ratio (95% CI)	p-value
1. Age ≤ 30 years (n=115)			
< 6 weeks	58 (95.10)	6.767 (1.83-25.09)	0.002*
≥ 6 weeks	40 (74.07)		
2. Age > 30 years (n=80)			
< 6 weeks	31 (81.58)	2.725 (0.97-7.63)	0.052
≥ 6 weeks	26 (61.90)		
3. Employment status was informal worker (n=116)			
< 6 weeks	75 (94.94)	2.920 (0.94-11.63)	0.113
≥ 6 weeks	32 (86.49)		
4. Employment status was formal worker (n=79)			
< 6 weeks	14 (70.00)	1.716 (0.58-5.09)	0.327
≥ 6 weeks	34 (57.63)		

*Statistical significance

Further analysis showed that the early postpartum period was associated with a decrease in the mean WAI to 31.60 ± 4.60 in week 4 and to 29.57 ± 4.07 in week 5. After this period, improvement in work ability was evident. The average WAI increased to 35.41 ± 2.57 in week 7th and was slightly higher to 35.90 ± 3.81 in week 8th. Overall, the findings suggest that work ability tends to be lower during the mid-postpartum period (particularly around weeks 4th to 5th) and improve as time progresses toward weeks 7th and 8th. The performance of the logistic regression model was evaluated in terms of overall model significance and calibration. The omnibus test of model coefficients indicated that the model was statistically significant ($\chi^2 = 43.871$, $df = 10$, $p < 0.001$), suggesting that the independent variables collectively contributed to the prediction of poor work ability. Calibration was assessed using the Hosmer–Lemeshow goodness-of-fit test, which showed no significant difference between observed and predicted outcomes ($\chi^2 = 4.787$, $df = 8$, $p = 0.780$), indicating a good model fit.

Discussion

The present study achieved the predetermined sample size as per the methodology. A total of 195 postpartum women were recruited (99 in the < 6 weeks group and 96 in the ≥ 6 weeks group), consistent with the calculated sample size for estimating the mean with a 95% confidence interval and a 20% margin of error. Therefore, the sample size was sufficient to provide adequate statistical precision and support the interpretation of the findings. Because

the study met its methodological requirements for sample adequacy, the results can be interpreted reliably in relation to the stated objectives.

This study also has notable strengths that address several limitations. It employed a standardized, internationally recognized instrument (the Work Ability Index), achieved an adequate sample size for estimating means, and examined occupational, personal, and organizational factors within a clearly defined conceptual framework. Furthermore, this study addresses an important gap in public and occupational health by focusing on postpartum work ability among informal workers in Thailand, a population that is often underrepresented in research.

A poorer WAI was more common in the postpartum group up to 6 weeks postpartum (89.9% vs. 68.8%). In addition, active diseases were also more common in the <6 weeks group, including wound pain (34.3% vs. 11.5%), sleep deprivation (60.6% vs. 43.8%), and postpartum depression (22.2% vs. 12.5%). These outcomes are supported by the physiological changes described in other studies, which note that maternal health may return to normal at approximately 6 weeks⁴ or later for heavier workloads or in cases requiring greater muscle strength¹⁴. However, the duty of raising children may continue, including breastfeeding, which can pose a health disadvantage (both physical and psychological) compared to women without this responsibility. Following Ilmarinen’s principle, the more health problems, the poorer the WAI¹⁰. Consequently, the postpartum women in the <6 weeks group had a poorer WAI. Likewise, the same reasoning

applies to the association with a poorer WAI in both groups compared with that of normally active young adults¹⁵.

The data showed that factors contributing to poorer WAI included age, education level, employment status, multiple demanding tasks, and work duration. The ≤ 30 -year age group was classified as the early-career group¹⁶, which is characterized by establishing stability in their jobs, while the > 30 -year age group (mid-career group¹⁶) had more control over their jobs and might have subordinates. As a result of the research, the lower age group had a higher OR at 2.950 (95%CI: 1.15-7.55). For education level (below a bachelor's degree) and employment status (informal worker), these factors also reflected poorer job control and poorer self-awareness regarding health issues. Motherhood requires both physical and psychological demands; the lower self-control task poses greater challenges for mothers raising children or breastfeeding during working hours¹⁷; for the same demanding requirement, multiple demanding tasks (both physical and cognitive) may increase the maternal burden more than a single demanding task does.

Some factors might have led to controversial results regarding the proportion of *2-month expectation attitude and mastitis/breast engorgement status*. The *2-month expectation if the situation remains unchanged* was affected by hope, current resources, and current ability. The discrepancy between higher perceived health coping and poorer WAI in the < 6 weeks group may reflect optimism bias or shorter exposure to work-related strain. Even though the burden in the first 6 weeks was heavier, the mothers had been exposed to work-related strain for a shorter duration; therefore, they had greater hope and the ability to buffer against the effects of obstacles¹⁸. However, the mothers in the ≥ 6 weeks group encountered longer tasks, which associated with more mental and physical strain in coping with the burden based on true available resources and true health status¹⁹. For mastitis/breast engorgement, the < 6 weeks group had a higher proportion because the structure of the workforce was predominantly informal workers, contributing insufficient time allowance for breastfeeding, which associated with further complications, such as mastitis²⁰, whereas the ≥ 6 weeks group was predominantly employed in the workplace; therefore, the workplace might have a breastfeeding corner, the workers might not delay breastfeeding, and as a result, they could reduce the major risk of breast engorgement or other complications²¹.

Subgroup analysis showed that workers aged > 30 years, regardless of formal or informal status, had higher odds of poor WAI when working before 6 weeks postpartum; however, this did not reach statistical significance. This could show that the consistency of working earlier might be associated with poorer WAI.

Even though the relationship between the working period and WAI score progression was explored, it showed a drop in WAI score from the 4th to 5th week. The reason was that this study was conducted on participants visiting the hospital. It was found that participants who responded at a later period within a certain subgroup might have had late visits or missed appointments, which was the primary reason for the inability to obtain clinical benefit²². They would lose opportunities for postpartum disease screening or management²³. These factors associated with medical and psychosocial complications of disease, reduced patients' quality of life, and wasted health care resources²⁴.

This study has several limitations. Firstly, the findings may introduce selection bias because it was not fully generalizable to all postpartum women, particularly those living in rural areas or outside hospital-based settings, because tasks in rural areas were predominantly agricultural²⁵, which involved more physical and strenuous work. However, social support differed from that in urban areas because the rural population tended to live in larger families²⁶. Secondly, the timing of the WAI test was based on patient visits; visits occurring later than the primary appointment might reflect personal problems rather than chronological health improvement.

Conclusions

Consistent with the conceptual framework, work ability in the postpartum period appears to be influenced by the number of days of recovery. The WAI of postpartum mothers who worked before 6 weeks was 30.51 ± 4.43 , lower than the mean WAI of 35.30 ± 3.44 for those who worked at 6 weeks or more ($p < 0.001$). The factors associated with poor WAI among postpartum mothers included working before 6 weeks, being an informal worker, and being aged 30 years or younger. When analyzing informal and formal workers separately and across age groups ≤ 30 years and > 30 years, working before 6 weeks was associated with poorer WAI. These results reaffirm that postpartum mothers who worked before 6 weeks had a significantly lower mean WAI than those who worked at 6 weeks or later, and that working before 6 weeks postpartum was a major factor associated with poor work ability. Finally, the findings suggest that early working without adequate structural support places women at high risk of functional decline. Policies that extend maternity protection, particularly for informal workers, and workplace interventions that reduce workload demands during the early postpartum period may substantially improve work ability outcomes.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Khon Kaen University Ethics Committee for Human Research (protocol code 56/2567 (No. HE671619)); approval date: February 25, 2025). Informed consent was obtained from all participants or their legal representatives.

Acknowledgements

The authors thank (a) the participants for their valuable time and cooperation in the study and (b) Mr. Bryan Roderick Hamman for assistance with the English-language presentation of the manuscript.

References

1. World Health Organization. Maternity protection compliance with international labour standards [Internet]. Geneva: World Health Organization; [cited 2026 Feb 15]. Available from: <https://www.who.int/data/nutrition/nlis/info/maternity-protection-compliance-with-international-labour-standards>.
2. Ministry of Labour (TH). Labour Protection act, B.E. 2541 (1998) [Internet]. 1998 [cited 2024 Mar 26]. Available from: https://www.labour.go.th/attachments/article/47756/Labour_Protection_Act_BE2541.pdf.

3. International Labour Organization. Care at work: Investing in care leave and services for a more gender equal world of work [Internet]. Geneva: International Labour Organization; 2022 [cited 2026 Feb 15]. Available from: https://www.ilo.org/wcmsp5/groups/public/-/dgreports/---gender/documents/publication/wcms_838655.pdf.
4. Hyman MH, Melhorn JM, Talmage JB, et al. AMA guides to the evaluation of work ability and return to work. 2nd ed. Chicago (IL): American Medical Association; 2011. p. 389-95.
5. Pongpramorn S. Maternal return to work within 6 months after delivery at Nopparat Rajthanee Hospital [master's thesis]. Bangkok: Srinakharinwirot University; 2013. Available from: <https://ir.swu.ac.th/server/api/core/bitstreams/4f9e957a-920c-487e-aded-9b8dd8b638e9/content>.
6. Han WJ, Ruhm CJ, Waldfogel J, et al. The timing of mothers' employment after childbirth. *Mon Labor Rev* 2008;131(6):15-27.
7. Saurel-Cubizolles MJ, Romito P, Escriba-Aguir V, et al. Returning to work after childbirth in France, Italy, and Spain. *Eur Sociol Rev* 1999;15(2):179-94. doi:10.1093/oxfordjournals.esr.a018259.
8. National Statistical Office (TH). Labour statistics indicators, 2014 (2557) [Internet]. Bangkok: National Statistical Office; 2014 [cited 2026 Feb 3]. Available from: https://www.nso.go.th/nsoweb/downloadFile/stat_impt/ik/file_xls_th.
9. National Statistical Office (TH). The informal labour situation in 2023: Approximately half work in the agricultural sector, and informal employees earn lower wages than formal workers [Internet]. 2023 [cited 2024 Apr 18]. Available from: https://www.nso.go.th/nsoweb/storage/upload_file/2023/20231214120252_84926.pdf.
10. Gould R, Ilmarinen J, Järvisalo J, et al., editors. Dimensions of work ability: Results of the Health 2000 survey [Internet]. Helsinki: Finnish Centre of Pensions; 2008 [cited 2026 Feb 6]. Available from: https://www.julkari.fi/bitstream/handle/10024/78055/dimensions_of_work_ability_7.pdf.
11. Finnish Social Science Data Archive. FSD2580 Itäsuomalaiset poliisit 1995-1998: terveys ja työolot - kyselylomake [Internet]. Tampere: University of Tampere; [cited 2026 Feb 15]. Available from: https://services.fsd.tuni.fi/catalogue/FSD2580/PIP/quF2580_i_fin.pdf.
12. Department of Disease Control (TH). Work ability index assessment form [Internet]. Nonthaburi: Department of Disease Control, Ministry of Public Health; [cited 2026 Feb 15]. Available from: <http://kfb.ddc.moph.go.th/dataentry/handbook/form/101>.
13. Career path progression: Junior to senior level [Internet]. Bangkok: Carenett Consultancy Recruitment; [cited 2026 Apr 20]. Available from: <https://www.carenett.one/tips/detail/2>.
14. Santos MLDS, Macêdo SGGF, Fernandes J, et al. Muscle strength during pregnancy and postpartum in adolescents and adults. *PLoS One* 2024;19(3):e0300062. doi:10.1371/journal.pone.0300062.
15. Kujala V, Remes J, Ek E, et al. Classification of work ability index among young employees. *Occup Med (Lond)* 2005;55(5):399-401. doi:10.1093/occmed/kqi075.
16. Vilela NG, Casado T. Career stages in management studies: A systematic review of scientific production from 2011 to 2020. *Rev Gest (Sao Paulo)* 2021;30(1):62-77. doi:10.1108/REGE-02-2021-0018.
17. Horwood C, Surie A, Haskins L, et al. Attitudes and perceptions about breastfeeding among female and male informal workers in India and South Africa. *BMC Public Health* 2020;20(1):875. doi:10.1186/s12889-020-09013-9.
18. Duncan AR, Jaini PA, Hellman CM. Positive psychology and hope as lifestyle medicine modalities in the therapeutic encounter: A narrative review. *Am J Lifestyle Med* 2020;15(1):6-13. doi:10.1177/1559827620908255.
19. Taylor SE, Stanton AL. Coping resources, coping processes, and mental health. *Annu Rev Clin Psychol* 2007;3:377-401. doi:10.1146/annurev.clinpsy.3.022806.091520.
20. Brugaillères P, Deguen S, Lioret S, et al. Maternal employment characteristics as a structural social determinant of breastfeeding after return to work in the European region: A scoping review. *Int Breastfeed J* 2024;19(1):38. doi:10.1186/s13006-024-00643-y.
21. Puapornpong P, Manolerthewan W, Raungrongmorakot K, et al. Factors effecting on breastfeeding success in infants up to 6 months of age in Nakhon Nayok province. *J Med Health Sci* 2009;16(3):117-23. (in Thai)
22. Dunbar-Jacob J, Erlen JA, Schlenk EA, et al. Adherence in chronic disease. *Annu Rev Nurs Res* 2000;18:48-90.
23. Wilcox A, Levi EE, Garrett JM. Predictors of non-attendance to the postpartum follow-up visit. *Matern Child Health J* 2016;20(Suppl 1):22-7. doi:10.1007/s10995-016-2184-9.
24. World Health Organization. Adherence to long-term therapies: Evidence for action [Internet]. Geneva: World Health Organization; 2003 [cited 2026 Mar 3]. Available from: <http://iris.who.int/server/api/core/bitstreams/121c6b73-8651-442f-9560-e3b1d9c8a75c/content>.
25. World Bank. Thailand rural income diagnostic: Challenges and opportunities for rural farmers [Internet]. Washington (DC): World Bank; 2022 [cited 2026 Mar 3]. Available from: <https://www.worldbank.org/en/country/thailand/publication/thailand-rural-income-diagnostic-challenges-and-opportunities-for-rural-farmers>.
26. Boonsanong W, Khampolsiri T, Chintanawat R. Factors related to loneliness among older persons living in extended families in rural areas. *Nurs J CMU* 2021;48(4):257-67. (in Thai)

ORIGINAL ARTICLE

Preventing Post-Inflammatory Hyperpigmentation After Laser Treatment of Solar Lentigines: A Randomized Controlled Trial of Corticosteroids and Brimonidine

Pakagamon Tumsutti¹, Salinee Rojhirunsakool², Nanticha Faromkao³, Saranya Khunkhet², Nathathai Prathumchat², Montree Udompataikul²

¹ Master of Science Program in Dermatology, Faculty of Medicine, Srinakharinwirot University

² Department of Dermatology, Faculty of Medicine, Srinakharinwirot University

³ Department of Preventive Medicine, Srinakharinwirot University

KEYWORDS

PIH prophylaxis; solar lentigines; topical corticosteroids; topical brimonidine; Q-switched Nd:YAG laser; erythema reduction

CORRESPONDING AUTHOR

Salinee Rojhirunsakool

Department of Dermatology, Faculty of Medicine, Srinakharinwirot University
114 Sukhumvit 23 Lane, Khlong Tan Nuea Sub-district, Wattana District, Bangkok 10110, Thailand
E-mail: salineer@g.swu.ac.th

Received: March 19, 2026

Revised: April 23, 2026

Accepted: April 29, 2026

ABSTRACT

Post-inflammatory hyperpigmentation (PIH) is a common adverse effect that occurs following laser removal of solar lentigines. Currently, the evidence of PIH prophylactic treatment is limited. This study aimed to determine the efficacy of multiple topical PIH prophylactic medications, including topical corticosteroids, topical corticosteroids under occlusion, and topical brimonidine. This study was a prospective, randomized, single-blinded, controlled, intra-individual, comparative study. Thirty-eight subjects with at least eight solar lentigines on their forearms were recruited to receive Q-switched Nd:YAG 532 nm laser treatment. The forearms were divided into four areas. The lesions in each area were randomly allocated to receive topical clobetasol, topical clobetasol under occlusion, topical brimonidine, or petrolatum jelly alone (control). The occurrence and intensity of PIH, degree of erythema, and improvement of lesions were evaluated at 2, 4, 8, and 12 weeks. Patient satisfaction was also assessed at the end of the trial. In this study, both topical clobetasol group and occluded clobetasol group showed significantly lower post-treatment erythema ($p=0.002$ and $p=0.004$) and higher patient satisfaction (both $p<0.001$) compared with the other groups. However, the occurrence and intensity of PIH and the improvement of lesions were not significantly different between all groups. In conclusion, topical clobetasol and clobetasol under occlusion can significantly minimize post-laser erythema and increase patient satisfaction; however, none of the medications can significantly prevent PIH.

Introduction

Solar lentigines are common age-related skin lesions¹. Q-switched (QS) Nd:YAG 532 nm laser is among the most effective treatment options. Although picosecond lasers have been shown to reduce the incidence of PIH following the treatment of pigmented lesions, QS lasers continue to be widely used due to their efficacy and affordability. However, post-inflammatory hyperpigmentation (PIH) was frequently reported in previous studies ranging from 8.47 to 47%²⁻⁷. Based on a previous study, PIH was observed in 25% of the lesions in Thai patients who had skin phototypes III-IV and underwent laser treatment for removal of solar lentigines on the forearms using a 532 nm QS Nd:YAG, 3-mm spot size and energy fluence of 1.4-1.7 J/cm². Meanwhile, a study in 193 Asians with skin phototypes III-V, who received 532 nm QS Nd:YAG irradiation for removing facial solar lentigines, reported that PIH developed in 23.18% of the lesions that were treated aggressively (very obvious immediate whitening of lesions), whereas the rate of PIH in the group that received mild irradiation (slight immediate whitening

of lesions) was 8.47%³. Another retrospective study reported that PIH occurred in 47% of patients after QS Nd:YAG 532 nm laser treatment of senile lentigines with a 3 mm spot size and energy fluence of 0.8-1.2 J/cm² in patients who had skin phototypes II-IV⁷. The wide range of incidences may occur due to several factors including the difference in skin phototypes of the patients, laser parameters, clinical endpoints, and location of the lesions⁸. The high incidence of PIH following QS laser treatment of solar lentigines remains a significant concern in Asian skin.

In clinical practice, a petrolatum-based ointment is commonly used for topical post-laser wound management to help maintain a moist environment to promote wound healing⁹. Various strategies have also been used to reduce the occurrence of PIH after laser therapy. Topical corticosteroids are among the prophylactic treatments that have been used, and occasionally, the medications are applied under occlusion to enhance absorption and provide a soothing sensation after treatment^{9,10}. The anti-inflammatory effect of topical corticosteroids may benefit the prevention of PIH

after laser therapy. Additionally, the vasoconstrictive effect may reduce post-treatment erythema. However, current evidence is limited, and further study is needed to determine the efficacy of topical corticosteroids in PIH prevention¹⁰.

Topical brimonidine gel, a selective α -2 adrenergic agonist that causes vasoconstrictive effect on dermal blood vessels, was also reported to have an anti-inflammatory effect in a previous study using mouse ear models^{10,11}. A few case reports have shown that topical brimonidine gel 0.33% may reduce erythema and PIH after laser therapy¹²⁻¹⁴.

Currently, the available evidence for PIH prophylaxis is sparse and controversial. Therefore, the aim of this study was to determine the efficacy of multiple topical PIH prophylactic treatments, including topical corticosteroids, topical corticosteroids under occlusion, and topical brimonidine, in reducing the incidence of PIH following 532 nm QS Nd:YAG laser treatment of solar lentigines.

Materials and Methods

Study Design and Participants

This was an experimental, prospective, randomized, single-blind, controlled, intra-individual comparative study. The clinical trial was approved by the Thai Clinical Trials Registry (TCTR20231024002). Thirty-eight participants who were at least 18 years old and had at least 8 solar lentigines on their forearms, diagnosed clinically by two dermatologists, were recruited at Srinakharinwirot University Skin Center. The lesions were to be located on four separate forearm areas: the left upper, left lower, right upper, and right lower forearms, with at least two lesions in each area. Before enrollment, all patients were informed about the study and given written consent. Patient history, including general history, past health history, current medications, and drug allergy, was obtained. Dermatologic examination was performed to assess the Fitzpatrick's skin phototypes (SPTs) of the patients. Patients who had active inflammatory diseases or infections at the site of treatment; who had a history of hypertrophic scars; who received topical retinoids, hydroquinone, or chemical peels, or other topical whitening agents at the site of treatment within 1 month; who underwent ablative laser therapy at the site of treatment within 6 months; who were allergic to topical analgesics, topical corticosteroids, topical brimonidine, petrolatum jelly, or other inactive ingredients of the medications; or who were pregnant or lactating women were excluded from the study.

Procedures

Solar lentigo lesions on each forearm were treated with a single session of a 532 nm QS Nd:YAG laser (MedLite C6; Hoya ConBio, Fremont, CA, USA), with a spot size of 3 mm and energy fluences of 1.0-1.8 J/cm² at a frequency of 2 Hz. The laser parameters were adjusted until the clinical endpoint of immediate whitening was achieved.

The lesions in each area were randomly assigned to PIH prophylactic treatments A, B, C, or D using a computer-generated block randomization with a block size of four. Treatment A consisted of applying topical clobetasol propionate 0.05% ointment (Dermovate®, GlaxoSmithKline UK, Middlesex, UK) twice daily for two days following laser therapy. Treatment B consisted of a single application of topical clobetasol propionate 0.05% ointment under occlusive dressings (Tegaderm; 3M, St Paul, MN, USA) for 24 h following laser. Treatment C was brimonidine tartrate 0.33% gel (Mirvaso®; Galderma, Lausanne, Switzerland), which was used 1 h before laser and once a day for 3 days afterwards. As a control, Treatment D consisted of applying petrolatum jelly four times daily for seven days. Petrolatum jelly was also applied to all lesions in Groups A, B, and C 4 times a day until 7 days after laser treatment to ensure comparably moist environment in all lesions. All participants and principal investigators were blinded to the treatment allocation.

The subjects were instructed to use broad-spectrum sunscreen with a sun protection factor of 50 and were advised to avoid sun exposure until the end of the study. Detailed instructions on how to apply each topical medication and the potential adverse reactions were given to the patients. Follow-up visits were scheduled at 2, 4, 8, and 12 weeks after treatment.

Outcome Measurement

Primary Outcome

The primary outcome of this study was the occurrence of PIH, which was defined as an increase in pigmentation in the treated area of each lesion after the crust had fallen off following a laser treatment. Blinded assessments were subjectively evaluated from the digital photographs at 2 weeks (or the day the crust completely fell off, if more than 14 days), and 4, 8, and 12 weeks by 2 dermatologists. The results were reported as "no PIH" or "presence of PIH". In cases of discordant results, both evaluators discussed the findings to reach a consensus.

Secondary Outcome

The intensity of PIH, degree of erythema, and improvement of lesions were subjectively assessed from the photographs by 2 dermatologists using 5-point grading scales at 2, 4, 8, and 12 weeks after laser treatment. Detailed definitions of each grading scale are provided in Figure 1.



5-point grading scale for intensity of PIH

Grade	Degree of erythema	Characteristics
0	No PIH	No darkening of pigmentation
1	Barely visible PIH	1-25% darkening
2	Mild PIH	26-50% darkening
3	Moderate PIH	51-75% darkening
4	Severe PIH	76-100% darkening

5-point grading scale for degree of erythema

Grade	Degree of erythema	Characteristics
0	No erythema	No skin redness
1	Barely visible erythema	1-25% skin redness
2	Mild erythema	26-50% skin redness
3	Moderate erythema	51-75% skin redness
4	Severe erythema	76-100% skin redness

5-point grading scale for improvement of lesions assessment

Grade	Improvement of lesions	Characteristics
0	None	No improvement or worsening of lesions
1	Poor improvement	1-25% improvement
2	Fair improvement	26-50% improvement
3	Good improvement	51-75% improvement
4	Excellent improvement	76-100% improvement

Figure 1 A 5-point grading scale for intensity of PIH, degree of erythema, and improvement of lesions.

The melanin and erythema indices of the selected lesions were measured using a Mexameter® MX18 (Courage + Khazaka Electronic GmbH, Cologne, Germany) at baseline, and 2, 4, 8, and 12 weeks after laser treatment. The measurements were performed 3 times on each lesion and the average melanin and erythema indices were calculated.

In addition, the duration of wound healing was recorded in the patient's logbook and the adverse effects were monitored at each visit. The patients were asked to evaluate their satisfaction at the end of the study using a 5-point grading scale.

Statistical Analysis

The sample size was calculated using the incidence of PIH following laser treatment in a previous study, which

found that the incidence of PIH on the side of the face treated with topical clobetasol was 40% (0.4), while the incidence of PIH on the control side was 75% (0.75)¹⁵. With 95% confidence and 80% power, each intervention required 72 lesions. With a 10% dropout rate, 80 lesions were required for each intervention.

The primary analysis was performed according to the per-protocol analysis. Linear mixed model was used to compare the continuous outcome between each method of PIH prophylaxis. Occurrence of PIH was analyzed by using multilevel mixed-effects Poisson regression. Multilevel mixed effects ordered logistic regression was used to compare the ordinal data of patient satisfaction between each group. Pearson's chi-square test and Fisher's exact test were used to compare categorical adverse effects

across groups. All statistical analyses were performed using Stata 17 (StataCorp., 2021; Stata Statistical Software, College Station, TX, USA: StataCorp LLC). $p < 0.05$ was considered as statistically significant.

Results

Baseline Characteristics

A total of 38 subjects were enrolled in the trial. However, one subject requested to withdraw from the study because of inability to follow up and one subject was discontinued due to failure to evaluate the primary outcome, therefore, a total of 36 subjects completed the study. There were three males and 33 females, and the median age of the patients was 58.5 years. The majority of the patients (58.33%) had skin type IV. There were no significant differences regarding baseline mean melanin indices between all the groups.

Occurrence of PIH

The occurrence of PIH is presented in Table 1. The inter-rater agreement between the two assessors was high, with $\kappa = 0.804$ (SE = 0.018; 95% CI, 0.769-0.839). The total incidence of PIH among all lesions was 68.75% (198/288 lesions). The incidence of PIH in the groups received topical clobetasol and clobetasol under occlusion, and brimonidine did not significantly differ from the control group ($p = 0.36$, 0.47, 0.89, respectively). Survival analysis revealed that PIH mostly occurred at week 4 and continued to develop until 12 weeks after laser treatment. Figure 2 shows clinical photographs of a representative case.

Table 1 Occurrence of PIH determined by dermatologists at week 2, 4, 8, and 12

Visit	Group A (n = 72)	Group B (n = 72)	Group C (n = 72)	Control (n = 72)	Overall incidence (n = 288)
Week 2	5	3	4	3	15
Week 4	34	31	26	23	114
Week 8	7	16	13	20	56
Week 12	4	2	5	2	13
Accumulative incidence	50	52	48	48	198
IRR (95% CI)	1.136 (0.86-1.49)	1.102 (0.84-1.43)	1.017 (0.79-1.30)	-	
p-value	0.36	0.47	0.89	-	

Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 h; Group C: topical brimonidine tartrate 0.33% gel 1 h before and 3 days after laser; Control: topical petrolatum jelly for 7 days. Values are number of lesions; IRR = Incidence Rate Ratio between the treatment and the control group. Significant differences at $p < 0.05$.

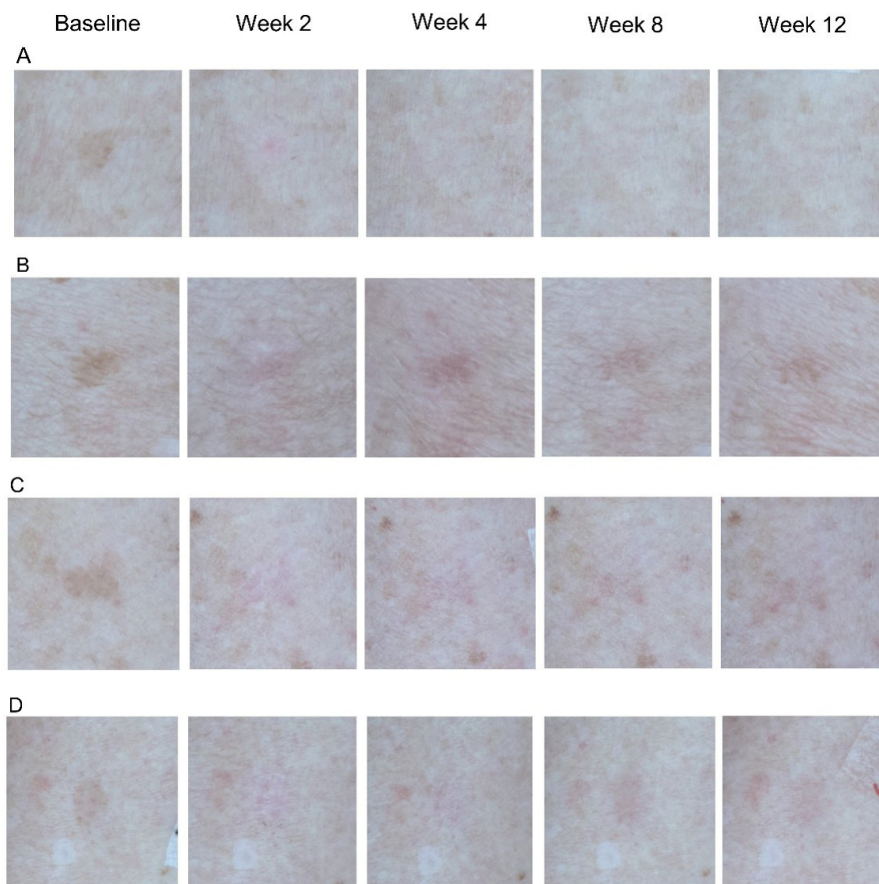


Figure 2 Clinical photographs after QS Nd:YAG 532 nm laser with (A) topical clobetasol propionate 0.05% ointment; (B) topical clobetasol propionate 0.05% ointment under occlusive dressings; (C) topical brimonidine tartrate 0.33% gel; (D) topical petrolatum jelly.

Intensity of PIH and Melanin Index

Figure 3 shows a subjective assessment of the intensity of PIH. Lesions in the topical clobetasol group had greater levels of PIH at weeks 4, 8, and 12, but the total severity

of PIH did not differ significantly between the treatment and control groups. Furthermore, there was no significant difference in melanin indices between all intervention groups and the control.

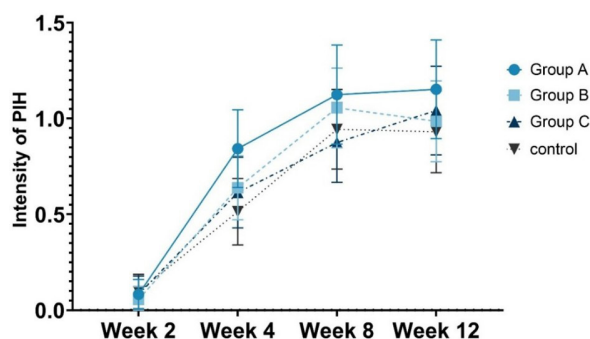


Figure 3 Intensity of PIH determined by the average grading of PIH for each intervention: Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 h; Group C: topical brimonidine tartrate 0.33% gel 1 h before and 3 days after laser; Control: topical petrolatum jelly for 7 days.

Degree of Erythema

The subjective evaluation of the degree of erythema is presented in Figure 4. The degree of erythema decreased over time following laser treatment. No erythema was observed in any lesions treated with topical clobetasol at weeks 8 and 12. A significantly lower degree of erythema was observed

in the lesions that received topical clobetasol and clobetasol under occlusion, compared to the control ($p=0.002$ and $p=0.004$, respectively). However, mean erythema indices by Mexameter were not significantly different between all groups.

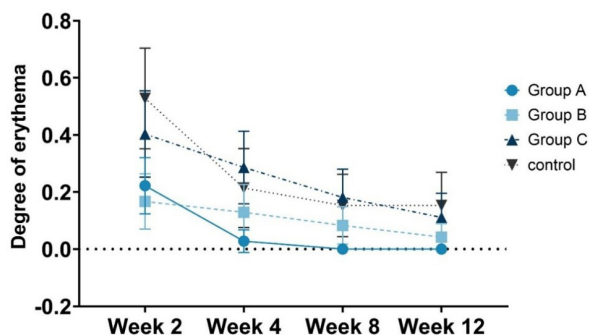


Figure 4 Degree of erythema determined by average grading of erythema of each intervention: Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 h; Group C: topical brimonidine tartrate 0.33% gel 1 h before and 3 days after laser; Control: topical petrolatum jelly for 7 days.

Improvement of Lesions

The assessment of improvement of lesions is as shown in Figure 5. There was no significant difference regarding the improvement of lesions between all groups.

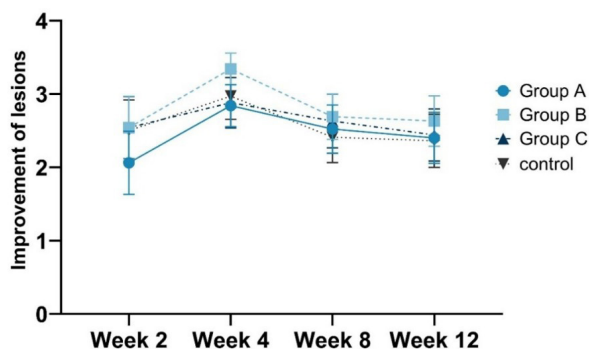


Figure 5 Improvement grading of solar lentigo by QS laser and topical intervention: Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 h; Group C: topical brimonidine tartrate 0.33% gel 1 h before and 3 days after laser; Control: topical petrolatum jelly for 7 days.

Patient Satisfaction

Overall satisfaction was significantly higher in all intervention groups compared with the control group ($p<0.001$), as shown in Figure 6. Several dimensions of satisfaction were also evaluated. With respect to lesion improvement, Groups A and C demonstrated significantly

greater improvement compared with the control group (both $p=0.008$). Satisfaction regarding convenience of use was higher in all intervention groups than in the control group ($p<0.001$). In addition, post-application sensation was rated significantly more favorably in all intervention groups ($p<0.001$).

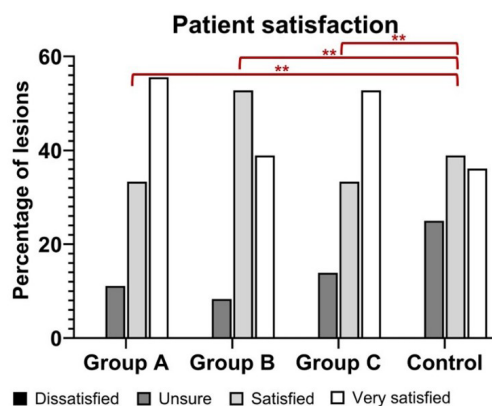


Figure 6 Patient satisfaction: Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 h; Group C: topical brimonidine tartrate 0.33% gel 1 h before and 3 days after laser; Control: topical petrolatum jelly for 7 days. *p*-value comparison between intervention groups and control, ***p*<0.001.

Adverse Reactions

Adverse reactions are listed in Table 2. Itching was significantly more prevalent in the lesions that were treated with topical brimonidine compared with the control (*p*=0.001). Moreover, three lesions (4.17%) in the topical

brimonidine group developed eczematous reactions that subsided within one week. There was no development of scarring or blistering after laser treatment. Possible side effects of topical corticosteroids, such as skin atrophy or acneiform eruptions, were not detected throughout the study.

Table 2 Adverse reactions and healing process

Parameter	Group A	Group B	Group C	Control	<i>p</i> -value
Adverse reactions, n (%)					
Itching	12 (16.67%)	8 (11.11%)	26 (36.11%)	12 (16.67%)	<0.01*
Dryness	18 (25.00%)	20 (27.28%)	22 (30.56%)	18 (25.00%)	0.872
Eczematous reactions	0 (0.00%)	0 (0.00%)	3 (4.17%)	0 (0.00%)	0.061
Healing process (Days)					
Pain, median	1	1	1	1	>0.999
Crusting, mean (SD)	18.93 (1.76)	18.79 (1.75)	18.49 (1.83)	18.86 (1.94)	0.478
Edema, median	1	1	1	1	>0.999
Erythema, median (IQR)	4 (3-7)	4 (3-7)	4.5 (3-7)	4.5 (3-7)	0.708

p-value = comparison of data between all groups; Significant differences at *p*<0.05.

Group A: topical clobetasol propionate 0.05% ointment twice daily for 2 days; Group B: single application of topical clobetasol propionate 0.05% ointment under occlusive dressings for 24 hours; Group C: topical brimonidine tartrate 0.33% gel 1 hour before and 3 days after laser; Control: topical petrolatum jelly for 7 days.

Duration of Wound Healing Process

The mean duration of pain and edema was 1 day after laser therapy in all groups. The overall mean duration of crusting was 18.77 ± 1.82 days and the mean duration of marked erythema was 4.92 ± 2.37 days. There was no significant difference regarding the duration of the wound healing between all groups, as shown in Table 2.

Discussion

In this clinical trial, topical clobetasol, topical clobetasol under occlusion, and topical brimonidine were not found to be significantly effective in reducing the incidence and intensity of PIH after laser treatment of solar lentigines, compared to petrolatum jelly alone. However, the reduction

of post-treatment erythema and patient satisfaction were significantly greater in lesions that were treated with topical clobetasol and topical clobetasol under occlusion. None of the interventions showed benefits over petrolatum jelly in terms of improvement of lesions and wound healing. Interestingly, we observed more side effects from topical brimonidine such as itching, dryness, and eczematous reaction in our study.

There are two major processes that result in PIH: cutaneous epidermal inflammatory response and pigmentary incontinence. A cutaneous epidermal inflammatory response can be generated by nonspecific thermal damage that occurred during laser therapy¹⁶. This reaction results in the release of eicosanoids, including prostaglandins E2 and D2, leukotrienes B4, C4, D4, and E4, and thromboxane B2,

which are generated from cell membranes after cutaneous inflammation. They contribute to the enlargement of melanocytes and increased proliferation of melanocyte dendrites, leading to an increase in melanin production and irregular distribution of melanin pigments to surrounding keratinocytes¹⁶⁻¹⁸. Meanwhile, pigmentary incontinence results from the damage of basal cell layer, which allows large amount of melanin to enter the dermis, followed by phagocytosis of melanin by dermal macrophages, which later become melanophages¹⁹.

Topical corticosteroids are indicated for the treatment of inflammatory skin conditions. Their mechanisms of action consist of anti-inflammatory, anti-proliferative, vasoconstrictive, and immunosuppressive effects. Corticosteroids inhibit the production of inflammatory mediators, including prostaglandins and leukotrienes¹⁶. Therefore, anti-inflammatory effects of topical corticosteroids may potentially benefit the prevention of PIH after laser therapy, and the vasoconstrictive properties may result in reducing the occurrence of post-treatment erythema. Occlusion by any method increases the temperature and hydration of the stratum corneum; therefore, it improves the absorption of topical corticosteroids²⁰. Moreover, the use of occlusion after laser therapy has been shown to improve patient-reported comfort and reduce post-treatment pain by limiting epidermal dehydration and mechanical irritation^{18,19}.

However, current evidence is limited and contradictory. Cheyasak et al.¹⁵ conducted a study using topical clobetasol propionate 0.05% ointment in the prevention of PIH after ablative fractional CO₂ laser resurfacing in 40 Asians with atrophic acne scars and SPTs IV. One side of the face was treated with clobetasol propionate twice a day for 2 days, while the other side was treated with petrolatum jelly four times per day. A significantly higher incidence of PIH was found on the side treated with petrolatum jelly (75%) compared with the side treated with topical clobetasol (40%)¹⁵. Meanwhile, another study on patients with facial Hori nevus who underwent 1064 nm QS Nd:YAG laser treatment found no significant difference regarding PIH incidence and melanin index between the lesions that received topical fucidic acid plus betamethasone valerate and fucidic acid alone²¹.

Topical brimonidine gel is a highly selective α -2 adrenergic agonist that causes a potent vasoconstrictive effect on dermal blood vessels with a diameter of less than 200 μ m. A few case reports have shown that topical brimonidine gel 0.33% may have a potential effect in reducing post-treatment erythema and PIH after laser therapy^{13,22,23}. The mechanism by which topical brimonidine reduces PIH after laser treatment is not clearly understood. Considering previous evidence reporting that endothelial cells can induce melanin synthesis, it is likely that the potential effect of topical brimonidine in preventing PIH might be associated with the decreased blood flow and vascularity²⁴.

In our study, topical clobetasol, along with topical clobetasol under occlusion and topical brimonidine, did not significantly reduce the occurrence of PIH compared with the control. We hypothesize that the difference in the primary lesions of the patients and laser-tissue interactions might affect the outcome of each piece of literature. The QS Nd:YAG 532 nm lasers generate short bursts and high-intensity radiation, which result in the rupture of melanosomes. Subsequently, the fragmented pigments are phagocytosed by melanophages, leading to gradual clearance of the

pigments²⁴. A previous study in which tissue biopsies were obtained immediately following the treatment of solar lentigines with a QS laser revealed that melanosomes were destroyed in clusters, with visible damage to the basement membrane²⁵. This might result in a greater amount of melanin being released from keratinocytes and melanocytes; thus, more hyperpigmentation occurs due to pigmentary incontinence, which may not be very responsive to topical corticosteroids. Furthermore, according to a previous study, inflammation in the skin can persist for 20 days following QS irradiation; therefore, the process of PIH development might continue during this period²⁶. Thus, a longer duration of anti-inflammatory treatment may be required to resolve this sustained inflammatory process.

The overall incidence of PIH following QS laser treatment of solar lentigines in this study was 68.75%, which is higher than most of the previous studies^{15,25,27-29}. This might result from the differences in the skin types of the patients and the locations of lesions; in most of the previous studies, the treatment was performed on the facial area, while in our study, the treatment was performed on the forearms. The higher density of sebaceous follicular units in the facial area might result in better wound healing²⁸. Additionally, some patients admitted that they did not have adequate sun protection after laser treatment, which could also affect the incidence of PIH²⁸.

In regard to post-laser erythema, topical clobetasol and topical clobetasol under occlusion were found to be effective in reducing erythema compared with the control group. This could result from the anti-inflammatory and vasoconstrictive effects of the treatment^{28,30}. However, the topical brimonidine group did not show improvement in erythema. These findings suggested that the vasoconstrictive effect alone may not effectively reduce post-treatment erythema or PIH, which arise from multifactorial processes. Furthermore, a higher incidence of adverse effects was observed in the topical brimonidine group. The lack of efficacy in reducing erythema could be explained by irritation or adverse effects, as itching was frequently reported, potentially contributing to persistent or increased erythema. Based on these findings, topical brimonidine is not recommended for post-laser wound care.

Interestingly, overall patient satisfaction was higher across all intervention groups. This is likely attributable to the fact that patient satisfaction reflects not only clinical efficacy, but also sensation after application and the convenience of use. Furthermore, the high satisfaction observed in the topical clobetasol under occlusion group is likely attributable to the ease of the single-application protocol and the soothing effect of the film dressing, which may help alleviate post-laser discomfort. Therefore, this approach may be beneficial for improving patient comfort and satisfaction after treatment.

The potential local side effects of topical corticosteroids, such as skin atrophy, striae, acne, and purpura, as well as the systemic side effects, were not detected throughout the study. The adverse reactions of topical corticosteroids usually occur following continuous treatment in the same region, especially with the application of high-potency topical corticosteroids on thin skin³⁰. According to the AAFP (American Academy of Family Physicians), the use of super-potent topical corticosteroids should not exceed 3 weeks, and shorter durations should be used when applied under occlusion³¹. Moreover, a previous study using a 2-day application of topical clobetasol propionate 0.05% ointment for PIH prophylaxis after fractional CO₂ on the face also

reported significantly lower occurrence of PIH with only 2 cases (5%) who developed acneiform eruption on the side treated with topical clobetasol and petrolatum jelly¹⁵. According to previous and recent studies, the application of potent topical corticosteroids for two days or single application under occlusion is considered safe. However, due to the non-significant benefit of topical corticosteroids in PIH prophylaxis, the author does not recommend the use of these treatments over petrolatum jelly.

There are some limitations of this study. First, the severity of PIH and erythema were assessed subjectively, which could lead to potentially inconsistent judgements. Second, despite being blinded to the treatment allocation, the patients might be able to notice the difference in texture and application method between each of the topical medications, potentially influencing performance bias. Third, given that the majority of participants in this study have skin type IV, the extrapolation of the findings across different phototype distributions should be interpreted with caution.

Conclusions

In this trial, topical clobetasol, topical clobetasol under occlusion, and topical brimonidine were not found to be significantly effective in reducing the occurrence and intensity of PIH after laser treatment of solar lentigines on the forearms, compared to the standard post-laser wound care (petrolatum alone). However, post-laser erythema was significantly lower following the application of topical clobetasol and topical clobetasol under occlusion. This correlates with the significantly better outcome perceived by the patients.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Human Research Ethics Committee of Srinakharinwirot University (protocol code SWUEC/F-352/2564; approval date: 24 February 2022). Informed consent was obtained from all participants or their legal representatives.

Acknowledgements

This study was funded by the faculty of medicine, Srinakharinwirot University, Thailand (Grant number 287/65). However, such funding did not influence the research design, interpretation of the data, or writing of the manuscript.

References

- Ortonne JP, Pandya AG, Lui H, et al. Treatment of solar lentigines. *J Am Acad Dermatol* 2006;54(5 Suppl 2): S262-71. doi:10.1016/j.jaad.2005.12.043.
- Vachiramon V, Panmanee W, Techapichetvanich T, et al. Comparison of Q-switched Nd: YAG laser and fractional carbon dioxide laser for the treatment of solar lentigines in Asians. *Lasers Surg Med* 2016;48(4):354-9. doi:10.1002/lsm.22472.
- Negishi K, Akita H, Tanaka S, et al. Comparative study of treatment efficacy and the incidence of post-inflammatory hyperpigmentation with different degrees of irradiation using two different quality-switched lasers for removing solar lentigines on Asian skin. *J Eur Acad Dermatol Venereol* 2013;27(3):307-12. doi:10.1111/j.1468-3083.2011.04385.x.
- Ho SG, Chan NP, Yeung CK, et al. A retrospective analysis of the management of freckles and lentigines using four different pigment lasers on Asian skin. *J Cosmet Laser Ther* 2012;14(2):74-80. doi:10.3109/14764172.2012.670707.
- Kang HJ, Na JI, Lee JH, et al. Postinflammatory hyperpigmentation associated with treatment of solar lentigines using a Q-Switched 532-nm Nd: YAG laser: A multicenter survey. *J Dermatolog Treat* 2017;28(5):447-51. doi:10.1080/09546634.2016.1254330.
- Wang CC, Sue YM, Yang CH, et al. A comparison of Q-switched alexandrite laser and intense pulsed light for the treatment of freckles and lentigines in Asian persons: A randomized, physician-blinded, split-face comparative trial. *J Am Acad Dermatol* 2006;54(5):804-10. doi:10.1016/j.jaad.2006.01.012.
- Satoh T, Etoh H, Kobayashi Y, et al. Postinflammatory hyperpigmentation after laser treatment of senile lentigine, comparing between Q-switched ruby laser and Q-switched frequency doubled Nd:YAG laser: A retrospective study. *J Japan Soc Laser Surg Med* 2016;37(1):24-9. doi:10.2530/jslsm.jslsm-37_0002.
- Wong ITY, Richer V. Prophylaxis of post-inflammatory hyperpigmentation from energy-based device treatments: A review. *J Cutan Med Surg* 2021;25(1):77-86. doi:10.1177/1203475420957633.
- Morales-Burgos A, Loosmore MP, Goldberg LH. Postoperative wound care after dermatologic procedures: A comparison of 2 commonly used petrolatum-based ointments. *J Drugs Dermatol* 2013;12(2):163-4.
- Rathi SK, D'Souza P. Rational and ethical use of topical corticosteroids based on safety and efficacy. *Indian J Dermatol* 2012;57(4):251-9. doi:10.4103/0019-5154.97655.
- Piwnica D, Rosignoli C, de Ménonville ST, et al. Vasoconstriction and anti-inflammatory properties of the selective α -adrenergic receptor agonist brimonidine. *J Dermatol Sci* 2014;75(1):49-54. doi:10.1016/j.jdermsci.2014.04.002.
- Bin Dakhil A, Shadid A, Altalhab S. Post-inflammatory hyperpigmentation after carbon dioxide laser: Review of prevention and risk factors. *Dermatol Reports* 2023;15(4):9703. doi:10.4081/dr.2023.9703.
- Braun SA, Artzi O, Gerber PA. Brimonidine tartrate 0.33% gel for the management of posttreatment erythema induced by laser skin resurfacing. *J Am Acad Dermatol* 2017;76(2):e53-5. doi:10.1016/j.jaad.2016.10.020.
- Chan HH, Manstein D, Yu CS, et al. The prevalence and risk factors of post-inflammatory hyperpigmentation after fractional resurfacing in Asians. *Lasers Surg Med* 2007;39(5):381-5. doi:10.1002/lsm.20512.
- Cheyasak N, Manuskiatti W, Maneeprasopchoke P, et al. Topical corticosteroids minimise the risk of postinflammatory hyper-pigmentation after ablative fractional CO₂ laser resurfacing in Asians. *Acta Derm Venereol* 2015;95(2):201-5. doi:10.2340/00015555-1899.
- Tomita Y, Maeda K, Tagami H. Melanocyte-stimulating properties of arachidonic acid metabolites: Possible role in postinflammatory pigmentation. *Pigment Cell Res* 1992;5(5 Pt 2):357-61. doi:10.1111/j.1600-0749.1992.tb00562.x.
- Taylor S, Grimes P, Lim J, et al. Postinflammatory hyperpigmentation. *J Cutan Med Surg* 2009;13(4):183-91. doi:10.2310/7750.2009.08077.

18. Tomita Y, Maeda K, Tagami H. Leukotrienes and thromboxane B2 stimulate normal human melanocytes in vitro: Possible inducers of postinflammatory pigmentation. *Tohoku J Exp Med* 1988;156(3):303-4. doi:10.1620/tjem.156.303.
19. Nordlund JJ, Abdel-Malek ZA. Mechanisms for post-inflammatory hyperpigmentation and hypopigmentation. *Prog Clin Biol Res.* 1988;256:219-36.
20. De Souza A, Strober BE. Principles of topical therapy. In: Goldsmith LA, Katz SI, Gilchrest BA, Paller AS, Leffell DJ, Wolff K, editors. *Fitzpatrick's Dermatology in General Medicine*. 8th ed. New York, USA: McGraw-Hill; 2012. p. 2641-54.
21. Uaboonkul T, Nakakes A, Ayuthaya PK. A randomized control study of the prevention of hyperpigmentation post Q-switched Nd:YAG laser treatment of Hori nevus using topical fucidic acid plus betamethasone valerate cream versus fucidic acid cream. *J Cosmet Laser Ther* 2012;14(3):145-9. doi:10.3109/14764172.2012.682066.
22. Hong JY, Lee HW, Park KY, et al. Brimonidine tartrate gel plus topical steroid for the prevention of laser therapy-related postinflammatory hyperpigmentation. *Dermatol Ther* 2018;31(5):e12657. doi:10.1111/dth.12657.
23. Lee SJ, Ahn GR, Seo SB, et al. Topical brimonidine-assisted laser treatment for the prevention of therapy-related erythema and hyperpigmentation. *J Cosmet Laser Ther* 2019;21(4):225-7. doi:10.1080/14764172.2018.1516887.
24. Regazzetti C, De Donatis GM, Ghorbel HH, et al. Endothelial cells promote pigmentation through endothelin receptor B activation. *J Invest Dermatol* 2015;135(12):3096-104. doi:10.1038/jid.2015.332.
25. Yamashita T, Negishi K, Hariya T, et al. In vivo microscopic approaches for facial melanocytic lesions after quality-switched ruby laser therapy: Time-sequential imaging of melanin and melanocytes of solar lentigo in Asian skin. *Dermatol Surg* 2010;36(7):1138-47. doi:10.1111/j.1524-4725.2010.01598.x.
26. Barua S. Laser-tissue interaction in tattoo removal by q-switched lasers. *J Cutan Aesthet Surg* 2015;8(1):5-8. doi:10.4103/0974-2077.155063.
27. Negishi K, Akita H, Matsunaga Y. Prospective study of removing solar lentigines in Asians using a novel dual-wavelength and dual-pulse width picosecond laser. *Lasers Surg Med* 2018;50(8):851-8. doi:10.1002/lsm.22820.
28. Davis EC, Callender VD. Postinflammatory hyperpigmentation: A review of the epidemiology, clinical features, and treatment options in skin of color. *J Clin Aesthet Dermatol* 2010;3(7):20-31.
29. Kim JY, Yang J, Huh G, et al. A split-face, single-blinded, randomized controlled comparison of 532 nm picosecond neodymium-doped yttrium aluminum garnet laser versus 532 nm q-switched neodymium-doped yttrium aluminum garnet laser in the treatment of solar lentigines. *Ann Dermatol* 2020;32(1):8-13. doi:10.5021/ad.2020.32.1.8.
30. Hengge UR, Ruzicka T, Schwartz RA, et al. Adverse effects of topical glucocorticosteroids. *J Am Acad Dermatol* 2006;54(1):1-15; quiz 16-8. doi:10.1016/j.jaad.2005.01.010.
31. Stacey SK, McEleney M. Topical corticosteroids: Choice and application. *Am Fam Physician* 2021;103(6):337-43.

ORIGINAL ARTICLE

Protective Effect of Mixed Probiotic Culture Against *Salmonella* Typhimurium Infection in Mice

Jirakit Supamanta^{1,2}, Chattramat Jaiyim², Kittiya Khongkool³, Chantanapa Chantarangkul², Praewpannarai Jamjuree², Kaikwa Wuttisan², Anongnard Kasorn⁵, Malai Taweechotipatr^{1,2}

¹ Applied Medical Science Program, Faculty of Medicine, Srinakharinwirot University

² Center of Excellence in Probiotics, Srinakharinwirot University

³ Biotechnology Program, Faculty of Science and Digital Innovation, Thaksin University

⁴ Department of Microbiology, Faculty of Medicine, Srinakharinwirot University

⁵ Department of Basic Medical Science, Faculty of Medicine Vajira Hospital, Navamindradhiraj University

KEYWORDS

Probiotics; *Salmonella* Typhimurium; intestinal infection; protective effect; gene expression

CORRESPONDING AUTHOR

Malai Taweechotipatr

Department of Microbiology, Faculty of Medicine, Srinakharinwirot University
114 Sukhumvit Road, Sukhumvit 23,
Wattana, Bangkok 10110, Thailand
E-mail: malai@g.swu.ac.th

ABSTRACT

Salmonella enterica serovar Typhimurium (*S. Typhimurium*) is a significant cause of foodborne illness and can lead to systemic infections, especially in immunocompromised individuals. Probiotics are known to enhance gut barrier function and modulate immune responses, thereby providing protection against enteric pathogens. This study evaluated the protective effect of a mixed probiotic culture-comprising *Bifidobacterium animalis* TA1, *Lactocaseibacillus paracasei* MSMC39-1, and *Limosilactobacillus reuteri* TF314-against *S. Typhimurium* infection in a murine model. Male ICR mice were categorized into three groups (n = 14 per group): control group (phosphate-buffered saline [PBS]), infected group (PBS + *S. Typhimurium*), and probiotic-treated group (probiotics + *S. Typhimurium*). Probiotics were administered orally for 30 days, then on days 31-33, consecutive challenge with *S. Typhimurium* infection was performed, and on day 34 blood and organs including; small intestine, colon, liver, and spleen were collected for microbiological culture, histopathological examination, Toll-like receptor gene expression analysis, and evaluation of tight junction proteins. Mice pretreated with probiotics showed no detectable *Salmonella* in the blood, liver, or spleen, and showed significantly lower intestinal bacterial loads compared to the infected group. Histological examination revealed reduced inflammation and preserved tissue architecture in the probiotic-treated group. Furthermore, probiotic treatment downregulated colonic expression of Toll-like receptor genes (*Tlr2* and *Tlr4*) and restored occludin levels, indicating improved intestinal barrier integrity. The findings indicate that the probiotic mixture effectively restricts *S. Typhimurium* infection and systemic dissemination through the modulation of immune signaling and the preservation of gut barrier integrity. This probiotic formulation represents a promising approach for the prevention of *Salmonella* infections.

Received: March 11, 2026

Revised: April 22, 2026

Accepted: May 2, 2026

Introduction

Gastrointestinal (GI) infections are a major global health burden, predominantly caused by bacterial, viral, or parasitic pathogens. These infections commonly present with symptoms such as diarrhea, abdominal cramps, nausea, vomiting, and, in severe cases, dehydration and systemic complications requiring hospitalization¹. While most GI infections are self-limiting, they can be life-threatening in neonates, immunocompromised individuals, and the elderly. Furthermore, recurrent or unresolved infections can predispose patients to long-term intestinal disorders². Among the bacterial pathogens, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) is a leading cause of foodborne enterocolitis worldwide³. As a Gram-negative, facultative intracellular pathogen belonging to the

Enterobacteriaceae family, *S. Typhimurium* invades the intestinal epithelium following ingestion of contaminated food or water and can disseminate systemically in vulnerable hosts⁴⁻⁶. Clinical manifestations range from mild gastroenteritis to severe complications such as intestinal bleeding, hepatic abscess, cholelithiasis, splenitis, and even septicemia. In some cases, infection may contribute to chronic inflammatory conditions and has been linked to an increased risk of colorectal cancer⁷⁻¹⁵. Although antibiotics are commonly used to treat severe infections, inappropriate or excessive use has accelerated the emergence of antibiotic-resistant *Salmonella* strains, highlighting the urgent need for alternative or adjunctive strategies for infection prevention¹⁶⁻²⁰.

Probiotics have emerged as promising candidates for preventing gastrointestinal infections due to their ability to modulate gut microbiota, reinforce mucosal immunity, and inhibit pathogen colonization. According to the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), probiotics are defined as “live microorganisms which, when administered in adequate amounts, confer a health benefit on the host.”²¹ Common probiotic genera include *Lactobacillus* and *Bifidobacterium*, which have been shown to promote intestinal homeostasis, enhance epithelial barrier integrity, suppress inflammation, and competitively inhibit pathogenic bacterial adherence and invasion^{22–26}. Several mechanisms underlie the protective effects of probiotics against pathogens, such as the production of short-chain fatty acids and lactic acid, competitive exclusion of pathogens for binding sites and nutrients, and modulation of Toll-like receptor-mediated signaling pathways (*Tlr*-mediated pathways). These properties are often strain-specific, necessitating in-depth evaluation of individual and mixed strains for efficacy in different host–pathogen models^{27–28}.

In this study, we evaluated the protective effect of a mixed probiotic culture consisting of three strains: *Bifidobacterium animalis* TA1 (*B. animalis* TA1), *Lactobacillus paracasei* MSMC39-1 (*L. paracasei* MSMC39-1), and *Limosilactobacillus reuteri* TF314 (*L. reuteri* TF314) against *S. Typhimurium* infection in a mouse model. The study aimed to assess the efficacy of this probiotic combination in preventing bacterial translocation to the blood and internal organs, as well as its effects on histopathological changes, *Tlr* expression, and intestinal tight junction integrity.

Materials and Methods

Preparation of Probiotics and *Salmonella Typhimurium*

Probiotic strains *B. animalis* TA1, *L. paracasei* MSMC39-1 and *L. reuteri* TF314 are listed in the Ministry of Public Health’s announcement as approved probiotic microorganisms for use in food by the Thai FDA. Frozen (–80 °C) stocks of probiotics were activated by culturing in de Man, Rogosa, and Sharpe (MRS) broth (MRS; HiMedia, India), incubated at 37 °C for 48 hours under anaerobic conditions (10% O₂, 10% CO₂, 80% N₂) using an anaerobic jar (Mitsubishi, Japan). The cultures were then subcultured on MRS agar three times and adjusted to a final concentration of the mixed formulation of 10⁹ CFU/mL before use in the experiment.

S. Typhimurium was maintained as frozen (–80 °C) stocks and cultured in Trypticase soy broth (TSB; HiMedia, India), incubated at 37 °C for 24 hours under aerobic conditions. The culture was then subcultured on Trypticase soy agar (TSA; HiMedia, India) three times and adjusted to a final concentration of 10⁸ CFU/mL prior to use in the experiment.

Animals and Experimental Design

Forty-two male ICR mice (*Mus musculus*), aged six weeks, were obtained from the National Laboratory Animal Center, Mahidol University (NLAC-MU), Bangkok, Thailand. Prior to the experiment, all animals underwent a one-week period of acclimatization. Mice were housed under standard hygienic conventional conditions (temperature 22 ± 2 °C, relative humidity 55 ± 10%, and a 12 h light/dark cycle) for the duration of the experiment. Animals were provided with a standard basal diet (3–5 g/mouse/day) and *ad libitum* access to water, and body weight was recorded daily.

Sample size estimation was performed using RStudio with the *pwr* package, applying a balanced one-way ANOVA power analysis ($k = 3$ groups, effect size $f = 0.5$, $\alpha = 0.05$, power = 0.8), resulting in an estimated minimum of 14 mice per group. Mice were randomly assigned to three groups ($n = 14$ per group):

- Control group: received 1 mL of sterile phosphate-buffered saline (PBS) via oral gavage on days 1–30.
- Infected group: received 1 mL of sterile PBS on days 1–30, followed by an oral challenge with *S. Typhimurium* SC2451 (1×10^8 CFU/mL/day) on days 31–33.
- Probiotic-treated group: received a mixed probiotic culture containing *B. animalis* TA1, *L. paracasei* MSMC39-1, and *L. reuteri* TF314 (1×10^9 CFU/mL/day) on days 1–30, followed by an oral challenge with *S. Typhimurium* on days 31–33.

After the challenge, animals were monitored daily for signs of toxicity, including behavior (gait, activity, lethargy), autonomic responses gastrointestinal symptoms (e.g., diarrhea). Local injuries and mortality were recorded. On days 34, mice were anesthetized using isoflurane and euthanized. Blood was collected via jugular vein puncture. Internal organs (liver, spleen, small intestine, colon), and intestinal fluid were harvested for further analysis.

Detection of *Salmonella*

The presence of *Salmonella* was assessed using Salmonella–Shigella (SS) agar, a selective and differential medium. Fecal samples were collected on days 0, 30, and 33 to monitor colonization. After euthanasia, homogenates of liver, spleen, blood, and intestinal fluid were prepared. Tissues were weighed (50–100 mg), washed twice with PBS (pH 7.2), and homogenized. Each sample (100 µL) was plated on SS agar and incubated at 37 °C for 18–24 hours. Colonies indicative of *Salmonella* (black centers) were counted to determine infection levels.

Histopathology Analysis

The liver, spleen, small intestine, and colon samples were fixed in 4% paraformaldehyde at 4 °C overnight and dehydrated for 1–2 h in a graded series of ethanol (10%, 20%, 50%, 95%, and 100%). The samples were then embedded in paraffin, and 3–5 µm sections of tissue were cut. The tissue sections were deparaffinized, cleared in xylene, and rehydrated in a graded series of ethanol (100%, 95%, and 70%) before staining with hematoxylin and eosin (H&E). Tissue sections were then observed under a light microscope (Olympus UC50, Japan).

For spleen samples, staining intensity and area were quantified using image analysis software ($n = 10$ randomly selected fields per group). Histopathological evaluation of the small intestine and colon was performed on H&E-stained sections ($n = 10$ randomly selected fields per group) using the Histopathological damage was quantified using a modified Roberts Histological Index (RHI), adapted to enhance sensitivity for murine models. The scoring system utilizes weighted parameters to reflect acute mucosal injury and barrier disruption, which are hallmarks of *Salmonella* induced colitis. The total score (0–33) was derived from the summation of four subscores: chronic inflammatory cell infiltration (0–3), neutrophils in the lamina propria (0–6), neutrophils in the epithelium (0–9), and epithelial erosion (0–15)⁴⁶.

Detection of Toll-Like Receptors by Real-Time PCR

Total RNA was extracted from colon tissue using TRIzol reagent (Invitrogen) according to the manufacturer's protocol, with DNase I digestion using the easy-spin™ Total RNA Extraction Kit. cDNA synthesis was performed with the Maxime RT PreMix Kit, followed by quantitative real-time PCR using RealMOD™ Green W2x qPCR mix (Intron, Korea). Specific primers used were:

- *Tlr2*: Forward 5'-AGACAAAGCGTCAAATCTCAG-3', Reverse 5'-GCATCACATGACAGAGACTCC-3'
- *Tlr4*: Forward 5'-TCCCTGCATAGAGGTAGTTCC-3', Reverse 5'-CAAGGGGTGAAGCTCAGATC-3'
- β -actin: Forward 5'-AGAGGGAATCGTGCCTGAC-3', Reverse 5'-CAATAGTGATGACCTGGCCGT-3'

A dissociation curve was generated to ensure that there was amplification of a single product and the absence of primer dimers. Reactions were performed in triplicate, and gene expression values were normalized using the geometric mean of β -actin. The quantification of relative mRNA expression was calculated using the comparative $\Delta\Delta C_t$ method. The relative change in target gene expression was calculated using the equation $2^{-\Delta\Delta C_t}$ where $\Delta\Delta C_t = \Delta C_t$ (calibrator) – ΔC_t (target).

Detection of Tight Junction Proteins by Immunohistochemistry

Colon tissues were fixed in 4% paraformaldehyde, dehydrated through a graded series of ethanol, embedded in paraffin, and sectioned at 5–10 μ m thickness. Sections were subjected to immunohistochemistry using the DAKO Envision System (Dako, VIC, Australia). Primary antibodies against ZO-1 and occludin (Lifespan Biosciences, WA, USA) were used to detect the expression of tight junction proteins. Staining intensity and area were quantified using image analysis software (n = 10 per group).

Statistical Analysis

Data were analyzed using GraphPad Prism 9. Normality was assessed prior to statistical analysis. For normally distributed data, comparisons among groups were performed using one-way ANOVA followed by Tukey's post hoc test. Non-normally distributed data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparisons post hoc test. A *p*-value of < 0.05 was considered statistically significant.

Results

Mixed Probiotic Culture Prevents Systemic Dissemination of *Salmonella Typhimurium*

Animals were monitored daily for clinical signs of toxicity following infection. On day 31, all groups challenged with *Salmonella Typhimurium* showed no observable abnormal clinical signs. On day 32, mice in the infected group exhibited a reduction in food intake, whereas normal feeding behavior was maintained in the probiotic-treated group. By day 33, mice in the infected group displayed evident clinical symptoms, including tremors, lethargy, ruffled fur, and body weight loss. In contrast, mice in the probiotic-treated group remained clinically normal throughout the observation period.

To assess the protective effects of the probiotic-treated group, bacterial translocation was evaluated by culturing blood, intestinal fluid, liver, and spleen samples on *Salmonella*–*Shigella* (SS) agar. The infected group showed 15, 920, 100, and 130 CFU/mL in the blood, intestine, liver, and spleen, respectively, which were significantly higher than those of the control group. In the probiotic-treated group, 190 CFU/mL was detected only in the intestine, with no systemic dissemination to blood or organs. The number of intestinal colonies was significantly lower than in the infected group but not significantly different from the control group (Figure 1).

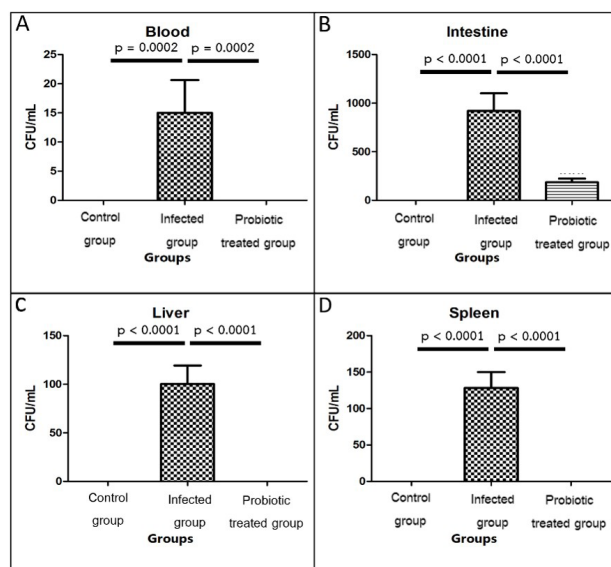


Figure 1 Number of colonies cultured from mouse samples: (A) blood, (B) intestinal fluid, (C) liver, and (D) spleen. One-way ANOVA followed by Tukey's post hoc test was used (n = 14 per group). Error bars represent the mean $\pm$ SD.

Probiotic Treatment Reduces Histopathological Damage in the Intestine and Organs

Small Intestine

Histopathological evaluation of small intestine tissues following *Salmonella* infection revealed varying degrees of intestinal damage. The control group exhibited mild villous blunting and minimal leukocyte infiltration (Figures 2A, 2D). In contrast, the infected group showed moderate villous

distortion, marked leukocyte accumulation in the lamina propria and submucosa, and epithelial erosion (Figures 2B, 2E). The probiotic-treated group displayed only mild villous distortion and low inflammatory cell infiltration (Figures 2C, 2F). Consistently, histological scoring demonstrated significantly reduced inflammation in the probiotic-treated group compared with the infected group (Figure 2G).

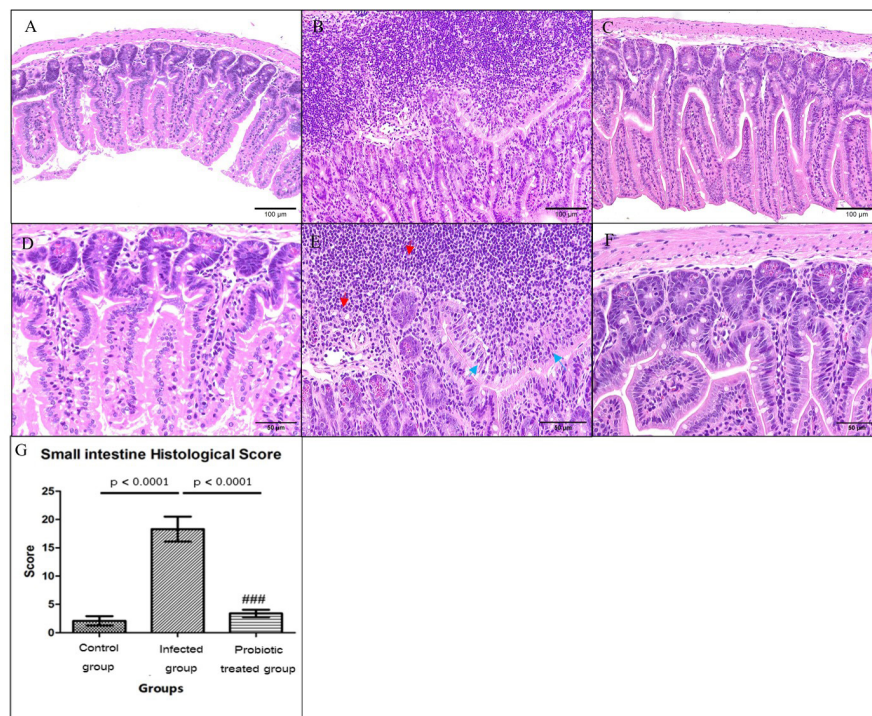


Figure 2 Representative histological images and histological scores indicating intestinal damage of the small intestine. (A) Control group longitudinal section; (B) Infected group longitudinal section; (C) Probiotic-treated group longitudinal section; (D) Control group longitudinal section; (E) Infected group longitudinal section; (F) Probiotic-treated group; longitudinal section. H&E stain; magnification A-C $\times 20$, D-F $\times 40$. Red arrows indicate leukocyte accumulation, and blue arrows indicate epithelial erosion. (G) Histological scores of the small intestine. One-way ANOVA followed by Tukey's post hoc test was used ($n = 10$ per group). Error bars represent the mean $\pm$ SD.

Colon

To further evaluate intestinal injury induced by *Salmonella* infection, histopathological assessment of large intestinal tissues was performed. The control group displayed intact crypt architecture, minimal leukocyte infiltration, and no signs of erosion (Figures 3A, 3D). Infected

mice demonstrated goblet cell depletion, crypt loss, and marked inflammation (Figures 3B, 3E). The probiotic-treated group showed only minor goblet cell loss and mild erosion (Figures 3C, 3F). Inflammation scores in the probiotic-treated group were significantly lower than those in the infected group (Figure 3G).

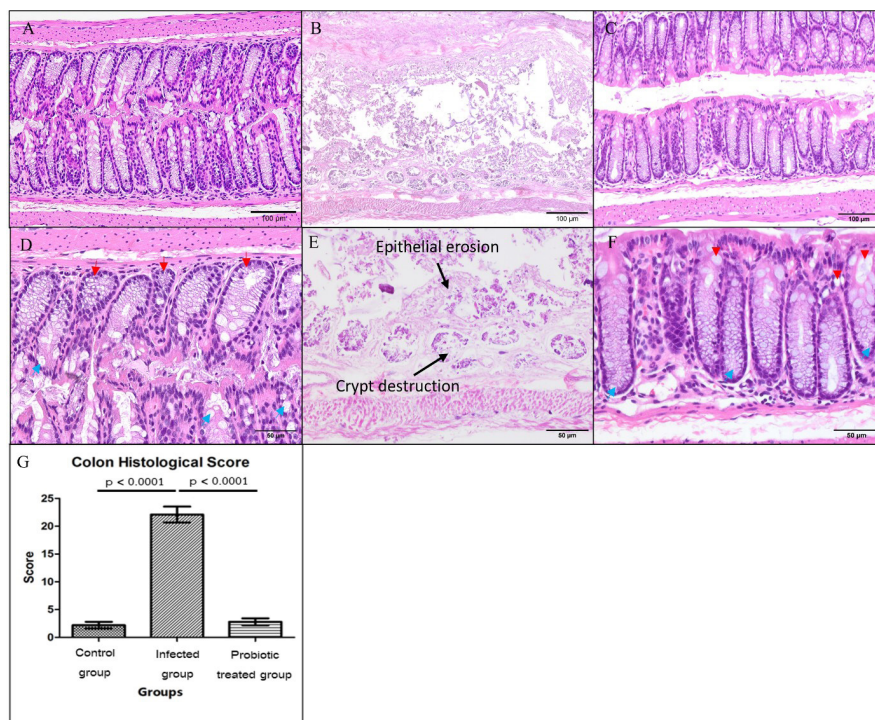


Figure 3 Representative histological images and histological scores indicating intestinal damage of the colon. (A) Control group longitudinal section; (B) Infected group longitudinal section; (C) Probiotic-treated group longitudinal section; (D) Control group longitudinal section; (E) Infected group longitudinal section; (F) Probiotic-treated group longitudinal section. H&E stain; magnification A-C $\times 20$, D-F $\times 40$. Red arrows indicate colon crypts and blue arrows indicate goblet cells. (G) Histological scores of the colon. One-way ANOVA followed by Tukey's post hoc test was used ($n = 10$ per group). Error bars represent the mean $\pm$ SD.

Liver

Liver sections from the control group showed no pathological alterations (Figures 4A, 4D). The infected group presented with hepatocellular necrosis, glycogen

accumulation, microgranuloma formation, and leukocyte infiltration (Figures 4B, 4E). These pathologies were markedly reduced in the probiotic-treated group (Figures 4C, 4F).

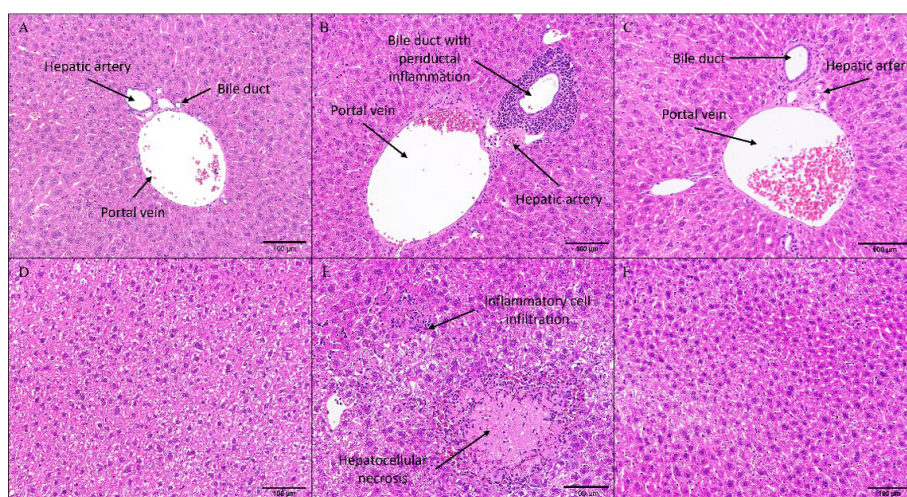


Figure 4 Representative histological images of the liver. (A) Portal triad of control group; (B) Portal triad of infected group; (C) Portal triad of probiotic-treated group; (D) Liver parenchyma of control group; (E) Liver parenchyma of infected group; (F) Liver parenchyma of probiotic-treated group. H&E stain; magnification $\times 20$.

Spleen

Spleens from control mice exhibited a normal white pulp area (~21.66%) with clear red–white pulp demarcation (Figures 5A, 5D). Infected mice showed marked splenic white pulp expansion (~81%), obliterating red pulp boundaries

(Figures 5B, 5D). The probiotic-treated group had a white pulp area (~23.29%) comparable to controls and significantly smaller than that of the infected group, indicating reduced splenic immune activation (Figures 5C, 5D).

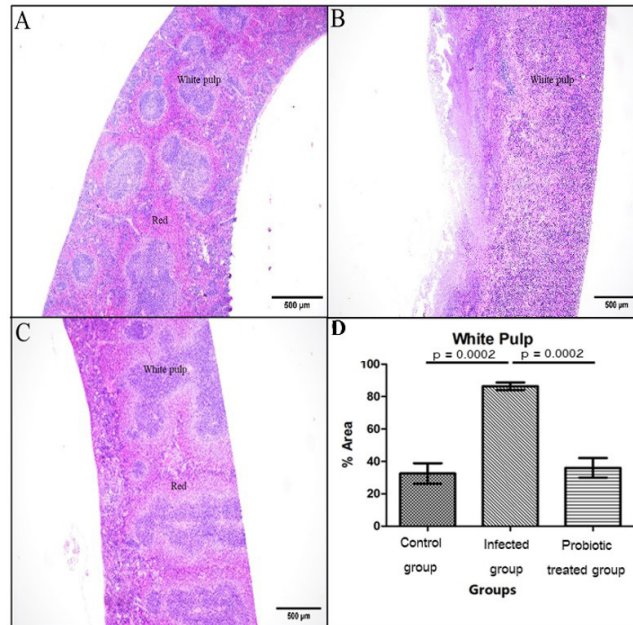


Figure 5 Representative histological images and percent area of white pulp of the spleen. (A) Control group; (B) Infected group; (C) Probiotic-treated group. H&E stain; magnification $\times 4$. (D) Percent area of white pulp in the spleen. One-way ANOVA followed by Tukey's post hoc test was used ($n = 10$ per group). Error bars represent the mean $\pm$ SD.

Probiotic Treatment Modulates Toll-Like Receptor (Tlr)

Immune responses were evaluated by assessing *Tlr2* and *Tlr4* mRNA expression in colon tissues using qPCR. *Tlr2* expression was slightly higher in the infected group, but the difference was not statistically significant compared

with the control. *Tlr4* expression was significantly higher in the infected group. Both *Tlr2* and *Tlr4* expression levels were significantly reduced in the probiotic-treated group compared to the infected group, indicating suppression of pathogen-induced *Tlr* activation (Figures 6A, 6B).

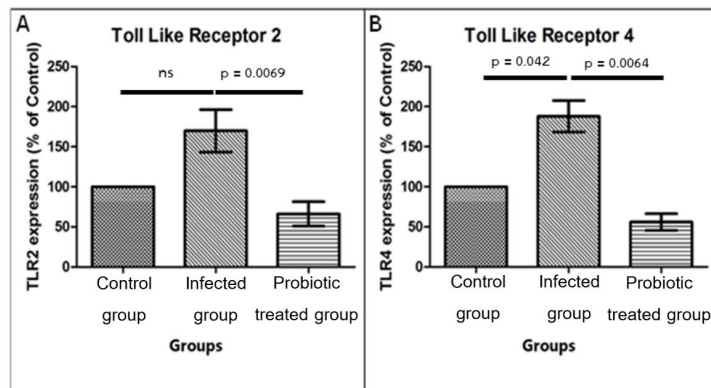


Figure 6 Relative mRNA expression levels of Toll-like receptors in the colon. (A) Toll-like receptor 2 (*Tlr2*) and (B) Toll-like receptor 4 (*Tlr4*). Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($n = 14$ per group). Error bars represent the mean $\pm$ SD.

Mixed Probiotic Culture Enhances Tight Junction Protein Expression

To investigate the effects of *Salmonella* infection on intestinal permeability, the expression of tight junction proteins was evaluated by immunohistochemical staining.

Occludin expression was significantly decreased in the infected group, whereas probiotic treatment significantly restored occludin levels (Figures 7A–7C, 7G). No statistically significant differences in ZO-1 expression were observed among the groups (Figures 7D–7F, 7H).

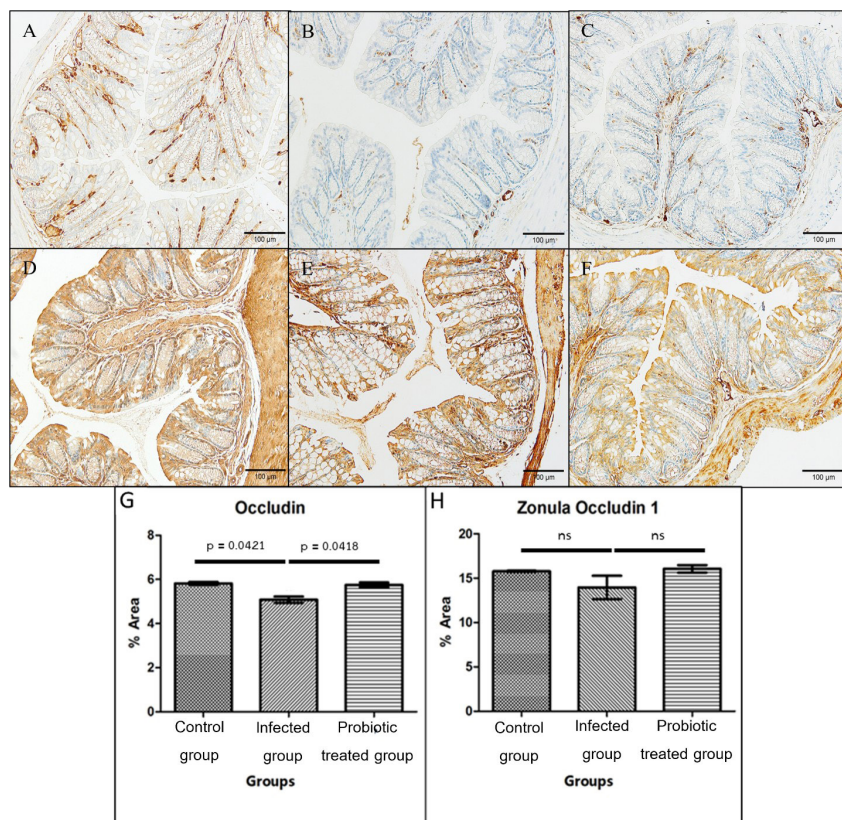


Figure 7 Tight junction proteins and percent area of tight junctions in the colon. (A) Occludin in the control group; (B) Occludin in the infected group; (C) Occludin in the Probiotic-treated group; (D) ZO-1 in the control group; (E) ZO-1 in the infected group; (F) ZO-1 in the Probiotic-treated group. Immunohistochemical staining; magnification $\times 20$. (G) Percent area of occludin in the colon; (H) Percent area of ZO-1 in the colon. Statistical significance was determined using the Kruskal–Wallis test followed by Dunn’s multiple comparison test ($n = 10$ per group). Error bars represent the mean $\pm$ SD.

Discussion

Probiotics possess various properties that are beneficial in preventing intestinal infections, such as lactic acid production, antimicrobial substance generation, and pathogen adhesion inhibition. Additionally, several studies have demonstrated that probiotics can enhance the immune system, reduce inflammation, and improve intestinal barrier function^{27–31}. In this study, we used a mixed probiotic culture *Bifidobacterium animalis* TA1, *Lactocaseibacillus paracasei* MSMC39-1, and *Limosilactobacillus reuteri* TF314 to test their protective properties against *Salmonella* Typhimurium infection in mice.

Salmonella is considered an important pathogen due to its ability to spread beyond the intestine and invade systemic organs such as the blood, liver, and spleen. In this study, we collected samples of blood, intestinal fluid, liver tissue, and spleen tissue. These samples were cultured on SS agar, a selective medium for isolating *Salmonella*, to

study the spread of the pathogen from the intestine to vital organs. The control group showed no *Salmonella* in any tested organs, indicating no contamination. In contrast, *Salmonella* were observed on SS agar plates cultured from the blood, intestinal fluid, liver, and spleen samples of the infected group. Importantly, the Probiotic-treated group, which received the mixed probiotic culture prior to *Salmonella* infection, exhibited no black colonies on SS agar derived from blood, liver, or spleen samples. Although *Salmonella* colonies were observed in intestinal fluid samples from this group, their number were significantly lower compared with the infected group. These findings indicate that the mixed probiotic culture effectively reduces intestinal colonization and prevents systemic invasion of *Salmonella*. This is consistent with previous studies demonstrating the protective effects of probiotics against pathogenic bacterial infections, including *Salmonella*^{20–23,26}.

To assess the effects of *Salmonella* infection, tissue samples from the small intestine, colon, liver, and spleen were examined microscopically for histopathological changes. No infection-related damage or abnormalities were observed in the control group. In the infected group, the small intestine showed severe inflammation with extensive leukocyte infiltration, villous damage, and tissue erosion³³. The colon exhibited tissue sloughing, goblet cell loss, and severe erosion³⁴. In the liver, extensive leukocyte infiltration around blood vessels, microgranuloma formation with multinucleated giant cells, and hepatocellular necrosis were observed³⁵. The spleen of infected mice exhibited marked white pulp expansion, reflecting lymphocyte proliferation in response to bloodstream infection. The excessive white pulp made the splenic boundaries indistinct³⁶. Histopathological examination of the intestine, liver, and spleen in the infected mice indicated that the infection caused significant damage to these organs.

In contrast, histopathological examination of the intestine, liver, and spleen from the mixed probiotic culture group showed mild inflammation. The organ damage was significantly reduced compared with the infected group. This suggests that the mixed probiotic culture can reduce the severity of *Salmonella* Typhimurium infection.

Several studies have highlighted the role of Toll-like receptors (*Tlrs*), which are immune system receptors critical for detecting and responding to infections in the body, particularly in the intestine. *Tlrs* interact with the Pathogen associated molecular pattern (PAMP) of pathogens, triggering the release of cytokines and chemokines, which are chemical signals that stimulate the movement of immune cells to the site of infection. *Tlrs* associated with *Salmonella* infection include *Tlr2* and *Tlr4*. *Tlr4* is specifically for lipopolysaccharides, a major component of the cell wall of *Salmonella*. Recent findings also indicate that *Tlr2* can bind to lipoproteins present on the *Salmonella* cell wall. Excessive immune activation can lead to a cytokine storm, resulting in the recruitment of a large number of leukocytes to the site of infection. These leukocytes can release proteolytic enzymes such as cathepsin G and elastase, which are capable of eliminating bacteria but may also cause damage to surrounding tissues^{12,16,40}. Additionally, certain cytokines, such as TNF- α , can induce cell death by binding to TNF receptor 1 (TNFR1) on the cell surface, thereby activating the caspase cascade and triggering apoptosis^{14,23}. These mechanisms represent essential immune responses for eliminating invading pathogens; however, when excessively activated, they can lead to inflammation and tissue damage¹²⁻¹⁴. In this study, the expression levels of *Tlr2* and *Tlr4* were increased in the infected group, which is consistent with previous findings indicating that *Tlr* expression is elevated in patients with bacterial infections and inflammatory bowel disease³⁷. In contrast, mice receiving the probiotic mixture showed lower *Tlr2* and *Tlr4* expression than the infected group, consistent with the known ability of probiotics to downregulate *Tlrs* and exert anti-inflammatory effects³⁸. Notably, mice treated with probiotics showed reduced *Tlr* expression and less severe *Salmonella* infection compared with infected mice. This is consistent with evidence that probiotics mitigate intestinal inflammation by modulating *Tlr* signaling³⁹. Alternatively, these benefits may result from lactic acid production, pathogen exclusion, and antimicrobial compound secretion.

Tight junctions are protein structures that regulate the space between cells, preventing the leakage of substances and blocking the entry of foreign agents such as pathogens or toxins from outside the body^{27,31}. When tight junctions are disrupted or become dysfunctional, it may lead to a condition known as "leaky gut," in which the intestinal epithelium is unable to effectively prevent the leakage of undesired substances into the body^{31,40}. This can allow toxins, pathogens, and other particles to pass into the bloodstream, potentially resulting in inflammation and inappropriate immune responses. In this study, we examined the levels of Zonula Occludens-1 (ZO-1), which is a key connector between tight junctions and the cell cytoskeleton, enhancing the stability of tight junctions and maintaining the integrity of cell-to-cell connections and occludin, a transmembrane protein that plays a role in connecting cells and regulating the passage of substances. Immunohistochemical analysis of the colon in mice revealed that the area of occludin was significantly reduced in the infected group compared with the control group. This finding is consistent with previous studies showing that *S. Typhimurium* can reduce the expression levels of tight junction proteins such as occludin, ZO-1, and claudin-1 in human intestinal epithelial cells (Caco-2) as well as in intestinal tissues of infected mice⁴⁰. However, mixed probiotic-treated mice exhibited a significantly greater occludin area than the infected group. This result aligns with studies indicating that *L. reuteri* ZJ617 and *Lactinaseibacillus rhamnosus* GG can mitigate tight junction damage induced by LPS in mice by upregulating the expression of occludin and claudin-3⁴¹. Additionally, *Bifidobacterium bifidum* has been shown to enhance tight junction integrity in Caco-2 cells by increasing the production of acetate and formate, which reduce inflammation and strengthen the intestinal epithelial barrier. It also helps prevent increased intestinal permeability induced by TNF- α ⁴². In contrast, no significant differences in ZO-1 area were observed among the three mouse groups.

The observed discrepancy between the significant reduction of occludin and the stability of ZO-1 levels may be attributed to their distinct structural roles and regulatory mechanisms within the tight junction complex. Occludin, as a transmembrane protein, is directly involved in forming the intercellular seal and is often more susceptible to rapid internalization or degradation triggered by pathogenic insults and inflammatory cytokines. In contrast, ZO-1 serves as a critical scaffolding protein that anchors the junctional complex to the actin cytoskeleton^{44,45}.

The resilience of ZO-1 in this study may further be explained by the activity of the *Salmonella* effector protein AvrA. Delivered into host cells via the Type III Secretion System (T3SS), AvrA acts as a deubiquitinase that stabilizes tight junction structures to facilitate bacterial persistence⁴³. Specifically, AvrA has been shown to maintain ZO-1 expression by modulating signaling pathways that inhibit the NF- κ B-mediated inflammatory response, thereby preventing the complete disassembly of the cytoplasmic plaque despite the loss of transmembrane proteins⁴³. Our findings are consistent with studies indicating that occludin is more susceptible to *S. Typhimurium*-induced disruption than ZO-1. Research by Liao et al. demonstrated that while *Salmonella* infection triggers the internalization of occludin, the presence of AvrA effectively preserves ZO-1 levels to maintain a degree of structural integrity⁴³. Similarly, Tahoun et al. observed that during the initial phases of enteric infection, transmembrane proteins like occludin undergo

rapid degradation due to increased mucosal permeability, whereas the intracellular protein ZO-1 remains relatively stable as a late-stage marker of junctional collapse⁴⁴. Therefore, the significant recovery of occludin in the probiotic-treated group, without a corresponding change in ZO-1, suggests that the protective effect of these probiotics is primarily focused on restoring the transmembrane 'seal' that was specifically targeted by the pathogen.

Despite the significant findings, several limitations of this study should be acknowledged. First, the research was conducted using a animal model, and while animal models are valuable for studying enteric infections, the physiological and immunological responses may not fully translate to human clinical settings. Second, this study evaluated the protective effects of the probiotic mixture against a single pathogen, *Salmonella* Typhimurium. Therefore, the findings may not be generalizable to all enteric infections. Future investigations should focus on evaluating this probiotic combination against a broader spectrum of enteric pathogens. Furthermore, well-designed clinical trials are necessary to validate the safety and efficacy of this probiotic formula in human populations for the prevention of salmonellosis and other gastrointestinal disorders.

Moving forward, future investigations should aim to expand the therapeutic scope of this triple-strain probiotic mixture by evaluating its efficacy against a broader spectrum of enteric pathogens, such as *Escherichia coli* and *Listeria monocytogenes*. Crucially, further studies should focus on the molecular mechanisms underlying the pathogen-induced degradation and probiotic-mediated restoration of occludin and ZO-1. This includes investigating how these probiotics prevent the internalization of occludin and whether they promote the assembly of the ZO-1 scaffolding complex to maintain barrier stability during infection. Furthermore, exploring the molecular crosstalk between these strains and the host's mucosal immune system particularly the TLR4/NF- κ B signaling pathways would provide deeper mechanistic insights. These future studies, potentially involving human intestinal organoids or clinical trials, will be crucial to validate the safety and translatability of this formula. Ultimately, the identified combination of *B. animalis* TA1, *L. paracasei* MSMC39-1, and *L. reuteri* TF314 holds significant promise as an antibiotic-free alternative for preventing foodborne infections and maintaining gut barrier integrity in high-risk populations.

Conclusions

This study demonstrates that a mixed probiotic culture consisting of *B. animalis* TA1, *L. paracasei* MSMC39-1, and *L. reuteri* TF314 effectively inhibits *Salmonella* Typhimurium colonization and systemic dissemination in a mouse model. The probiotic mixture significantly attenuated histopathological damage in the intestine, liver, and spleen. Notably, the treatment effectively downregulated the pathogen-induced of *Tlr4* and *Tlr2*, and selectively enhanced the expression of the transmembrane tight junction protein occludin, while ZO-1 levels remained relatively stable across all groups. These findings suggest that the probiotic combination confers protection primarily by suppressing the TLLR-mediated inflammatory response and reinforcing the epithelial 'seal' through occludin restoration, providing strong evidence for its use in preventing enteric infections and maintaining gut barrier integrity.

Ethical statement

All animal experiments were conducted in accordance with institutional and national ethical guidelines to ensure animal welfare through proper care practices. The study protocol was reviewed and approved by the Animal Ethics Committee of Srinakharinwirot University (approval number: COA/AE-015-2566).

Acknowledgements

This study was supported by the Faculty of Medicine and HRH Princess Maha Chakri Sirindhorn Medical Center, Srinakharinwirot University (Grant Number 143/2568), and by the Center of Excellence in Probiotics, Srinakharinwirot University (Grant Number 148/2569).

References

1. Burd EM, Hinrichs BH. Gastrointestinal infections. In: Molecular pathology in clinical practice. 2nd ed. Cham: Springer; 2016. p. 707-34. doi:10.1007/978-3-319-19674-9_50.
2. Fantry L. Gastrointestinal infections in the immunocompromised host. *Curr Opin Gastroenterol* 2000; 16(1):45-50. doi:10.1097/00001574-200001000-00008.
3. Brown EW, Bell R, Zhang G, et al. *Salmonella* genomics in public health and food safety. *EcoSal Plus* 2021;9(2):eESP00082020. doi:10.1128/ecosalplus.ESP-0008-2020.
4. Ferrari RG, Rosario DKA, Cunha-Neto A, et al. Worldwide epidemiology of *Salmonella* serovars in animal based foods: A meta-analysis. *Appl Environ Microbiol* 2019;85(14):e00543-19. doi:10.1128/AEM.00591-19.
5. Heymans R, Vila A, van Heerwaarden CAM, et al. Rapid detection and differentiation of *Salmonella* species, *Salmonella* Typhimurium and *Salmonella* enteritidis by multiplex quantitative pcr. *PLoS One* 2018;13(10):e0206316. doi:10.1371/journal.pone.0206316.
6. Cho JH. Successful endoscopic hemoclippping and conservative management for typhoid fever complicated by massive intestinal bleeding and acute pancreatitis: Case report. *Medicine (Baltimore)* 2019;98(31):e16521. doi:10.1097/MD.00000000000016521.
7. Wang X, Yang B, Ma S, et al. Lactate promotes *Salmonella* intracellular replication and systemic infection via driving macrophage M2 polarization. *Microbiol Spectr* 2023;11(6):e0225323. doi:10.1128/spectrum.02253-23.
8. Jiang L, Wang P, Song X, et al. *Salmonella* Typhimurium reprograms macrophage metabolism via T3SS effector SopE2 to promote intracellular replication and virulence. *Nat Commun* 2021;12(1):879. doi:10.1038/s41467-021-21186-4.
9. Johnson LA, Rodansky ES, Tran A, et al. Effect of abt-263 on intestinal fibrosis in human myofibroblasts, human intestinal organoids, and the mouse *Salmonella* typhimurium model. *Inflamm Bowel Dis* 2022;28(2):161-75. doi:10.1093/ibd/izab166.
10. van Elsland DM, Duijster JW, Zhang J, et al. Repetitive non-typhoidal *Salmonella* exposure is an environmental risk factor for colon cancer and tumor growth. *Cell Rep Med* 2022;3(12):100852. doi:10.1016/j.xcrm.2022.100852.
11. Shukla R, Shukla P, Behari A, et al. Roles of *Salmonella* typhi and *Salmonella* paratyphi in gallbladder cancer development. *Asian Pac J Cancer Prev* 2021;22(2):509-16. doi:10.31557/APJCP.2021.22.2.509.

12. Perez-Toledo M, Beristain-Covarrubias N, Pillaye J, et al. Discrete and conserved inflammatory signatures drive thrombosis in different organs after *Salmonella* infection. *Nat Commun* 2025;16(1):2356. doi:10.1038/s41467-025-57466-6.
13. Lamichhane B, Mawad AMM, Saleh M, et al. Salmonellosis: An overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistant infections. *Antibiotics* 2024;13(1):76. doi:10.3390/antibiotics13010076.
14. Wang R, Li S, Jia H, et al. Protective effects of cinnamaldehyde on the inflammatory response, oxidative stress, and apoptosis in liver of *Salmonella* Typhimurium-challenged mice. *Molecules* 2021;26(8):2309. doi:10.3390/molecules26082309.
15. Sivanandy P, Yuk LS, Yi CS, et al. A systematic review of recent outbreaks and the efficacy and safety of drugs approved for the treatment of *Salmonella* infections. *IJID Reg* 2024;14:100516. doi:10.1016/j.ijregi.2024.100516.
16. Crump JA, Sjölund-Karlsson M, Gordon MA, et al. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. *Clin Microbiol Rev* 2015;28(4):901-37. doi:10.1128/CMR.00002-15.
17. Xiang Y, Zhu K, Min K, et al. Characterization of a *Salmonella* enterica serovar typhimurium lineage with rough colony morphology and multidrug resistance. *Nat Commun* 2024;15(1):6123. doi:10.1038/s41467-024-50331-y.
18. Peng M, Salaheen S, Buchanan RL, et al. Alterations of *Salmonella* enterica serovar typhimurium antibiotic resistance under environmental pressure. *Appl Environ Microbiol* 2018;84(19):e01173-18. doi:10.1128/AEM.01173-18.
19. Mechesso AF, Park SC. Tylosin exposure reduces the susceptibility of *Salmonella* Typhimurium to florfenicol and tetracycline. *BMC Vet Res* 2020;16(1):22. doi:10.1186/s12917-020-2246-5.
20. Shaji S, Selvaraj RK, Shanmugasundaram R. *Salmonella* infection in poultry: A review on the pathogen and control strategies. *Microorganisms* 2023;11(11):2814. doi:10.3390/microorganisms11112814.
21. Jan T, Negi R, Sharma B, et al. Next generation probiotics for human health: An emerging perspective. *Heliyon* 2024;10(16):e35980. doi:10.1016/j.heliyon.2024.e35980.
22. Petrariu OA, Barbu IC, Niculescu AG, et al. Role of probiotics in managing various human diseases, from oral pathology to cancer and gastrointestinal diseases. *Front Microbiol* 2024;14:1296447. doi:10.3389/fmicb.2023.1296447.
23. Mazziotta C, Tognon M, Martini F, et al. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells* 2023;12(1):184. doi:10.3390/cells12010184.
24. Ladda B, Jantarassamee C, Pradidarcheep W, et al. Anti-inflammatory and gut microbiota modulating effects of probiotic *Lactobacillus paracasei* MSMC39-1 on dextran sulfate sodium-induced colitis in rats. *Nutrients* 2023;15(6):1388. doi:10.3390/nu15061388.
25. Zhang Y, Wang W. Probiotics in reducing obesity by reconfiguring the gut microbiota. *J Cent South Univ Med Sci* 2024;49(7):1042-51. doi:10.11817/j.issn.1672-7347.2024.240361.
26. Gut AM, Vasiljevic T, Yeager T, et al. *Salmonella* infection - prevention and treatment by antibiotics and probiotic yeasts: A review. *Microbiology (Reading)* 2018;164(11):1327-44. doi:10.1099/mic.0.000709.
27. Plaza-Diaz J, Ruiz-Ojeda FJ, Gil-Campos M, et al. Mechanisms of action of probiotics. *Adv Nutr* 2019;10(Suppl 1):S49-66. doi:10.1093/advances/nmy063.
28. Thoda C, Touraki M. Immunomodulatory properties of probiotics and their derived bioactive compounds. *Appl Sci* 2023;13(8):4726. doi:10.3390/app13084726.
29. Tenea GN, Gonzalez GL, Moreno JL. Probiotic characteristics and antimicrobial potential of a native *Bacillus subtilis* strain FA17.2 rescued from wild Bromelia sp. flowers. *Microorganisms* 2022;10(5):860. doi:10.3390/microorganisms10050860.
30. Gao J, Xu K, Liu H, et al. Impact of the gut microbiota on intestinal immunity mediated by tryptophan metabolism. *Front Cell Infect Microbiol* 2018;8:13. doi:10.3389/fcimb.2018.00013.
31. di Vito R, Conte C, Traina G. A multi-strain probiotic formulation improves intestinal barrier function by the modulation of tight and adherent junction proteins. *Cells* 2022;11(16):2617. doi:10.3390/cells11162617.
32. Han J, Aljahdali N, Zhao S, et al. Infection biology of *Salmonella* enterica. *EcoSal Plus* 2024;12(1):eesp00012023. doi:10.1128/ecosalplus.esp-0001-2023.
33. Bellido-Carreras N, Arguello H, Zaldivar-Lopez S, et al. *Salmonella* Typhimurium infection along the porcine gastrointestinal tract and associated lymphoid tissues. *Vet Pathol* 2019;56(5):681-90. doi:10.1177/0300985819843682.
34. Yan J, Racaud-Sultan C, Pezier T, et al. Intestinal organoids to model *Salmonella* infection and its impact on progenitors. *Sci Rep* 2024;14(1):15160. doi:10.1038/s41598-024-65485-4.
35. Gonzalez-Escobedo G, La Perle KM, Gunn JS. Histopathological analysis of *Salmonella* chronic carriage in the mouse hepatopancreatobiliary system. *PLoS One* 2013;8(12):e84058. doi:10.1371/journal.pone.0084058.
36. Roux CM, Butler BP, Chau JY, et al. Both hemolytic anemia and malaria parasite-specific factors increase susceptibility to nontyphoidal *Salmonella enterica* serovar typhimurium infection in mice. *Infect Immun* 2010;78(4):1520-7. doi:10.1128/IAI.00887-09.
37. Goodi GAK, Ghaima KK. Evaluation of gene expression levels of Tlr2, 4, and 5 genes in patients with chronic suppurative otitis media (CSOM) and relationship with bacterial infection. *SEEU J Public Health* 2024;295-302. doi:110.70135/seejph.vi.986.
38. Forsberg A, Abrahamsson TR, Björkstén B, et al. Pre- and postnatal administration of *Lactobacillus reuteri* decreases Tlr2 responses in infants. *Clin Transl Allergy* 2014;4:21. doi:10.1186/2045-7022-4-21.
39. Yao P, Tan F, Gao H, et al. Effects of probiotics on toll-like receptor expression in ulcerative colitis rats induced by 2,4,6-trinitro-benzene sulfonic acid. *Mol Med Rep* 2017;15(4):1973-80. doi:10.3892/mmr.2017.6226.
40. Kohler H, Sakaguchi T, Hurley BP, et al. *Salmonella enterica* serovar typhimurium regulates intercellular junction proteins and facilitates transepithelial neutrophil and bacterial passage. *Am J Physiol Gastrointest Liver Physiol* 2007;293(1):G178-87. doi:10.1152/ajpgi.00535.2006.

41. Cui Y, Liu L, Dou X, et al. *Lactobacillus reuteri* ZJ617 maintains intestinal integrity via regulating tight junction, autophagy and apoptosis in mice challenged with lipopolysaccharide. *Oncotarget* 2017;8(44):77489-99. doi:10.18632/oncotarget.20536.
42. Fernandez J, Guerra B, Rodicio MR. Esistence to carbapenems in non-typhoidal *Salmonella enterica* serovars from humans, animals and food. *Vet Sci* 2018;5(2):40. doi:10.3390/vetsci5020040.
43. Liao AP, Petrof EO, Kuppireddi S, et al. *Salmonella* type III effector AvrA stabilizes cell tight junctions to inhibit inflammation in intestinal epithelial cells. *PLoS One* 2008;3(6):e2369. doi:10.1371/journal.pone.0002369.
44. Moonwiriyaakit A, Pathomthongtaweetchai N, Steinhagen PR, et al. Tight junctions: From molecules to gastrointestinal diseases. *Tissue Barriers* 2023;11(2):2077620. doi:10.1080/21688370.2022.2077620.
45. Al-Sadi R, Boivin M, Ma T. Mechanism of cytokine modulation of epithelial tight junction barrier. *Front Biosci (Landmark Ed)* 2009;14(1):2765-78. doi:10.2741/3413.
46. Vespa E, D'Amico F, Sollai M, et al. Histological scores in patients with inflammatory bowel diseases: The state of the art. *J Clin Med* 2022;11(4):939. doi:10.3390/jcm11040939.

ORIGINAL ARTICLE

Comparative Analysis of Asthma Control Assessment Questionnaires in Children Using the Global Initiative for Asthma (GINA) Guidelines: A Prospective Cohort Study

Prapassinee Suntipraron

Department of Pediatrics, Nakhonpathom Hospital

KEYWORDS

pediatric asthma; asthma control; questionnaires; exacerbation; predictive validity

CORRESPONDING AUTHOR

Prapassinee Suntipraron

Department of Pediatrics, Nakhonpathom Hospital

196 Thesa Road, Phra Pathom Chedi Sub-district, Mueang Nakhon Pathom District, Nakhon Pathom 73000, Thailand

E-mail: prapassineesuntipraron@gmail.com

Received: January 29, 2026

Revised: April 24, 2026

Accepted: May 6, 2026

ABSTRACT

Assessing asthma control is essential for guiding treatment and preventing exacerbations in children. This prospective cohort study aimed to compare the agreement and diagnostic accuracy of three asthma control questionnaires: the Childhood Asthma Control Test (C-ACT), Asthma Control Test (ACT), and 6-item Asthma Control Questionnaire (ACQ-6) against the Global Initiative for Asthma (GINA) guideline and spirometry, and evaluated their predictive validity for 3-month exacerbations. A total of 186 children with physician-diagnosed asthma, aged 7–15 years, were enrolled. Children aged 7–11 years ($n=93$) completed the C-ACT, while those aged 12–15 years ($n=93$) completed the ACT. All participants ($n=186$) completed the ACQ-6. Statistical analyses included weighted kappa (k), Pearson correlation coefficients, ROC curves, and logistic regression analysis. Results: All three assessment tools demonstrated significant agreement with the GINA guidelines ($p<0.001$). ACT showed good agreement ($k=0.723$; 75.30%), while ACQ-6 and C-ACT showed moderate agreement ($k=0.449$; 55.91%, $k=0.539$; 65.59%, respectively). Regarding diagnostic accuracy for identifying uncontrolled asthma, ACT achieved the highest performance (AUC=0.901, cut-point ≤ 19), followed by ACQ-6 (AUC=0.830, cut-point ≥ 1.0) and C-ACT (AUC=0.774, cut-point ≤ 22). In terms of predictive validity for exacerbations within 3 months, patients classified as uncontrolled by ACT and ACQ-6 had significantly higher risks of exacerbation compared to the controlled / partly controlled status (aOR=14.308; $p=0.001$ and aOR=26.492; $p<0.001$, respectively). All three tools demonstrated significant predictive accuracy ($p<0.05$): ACT (AUC 0.879) and ACQ-6 (AUC 0.872) showed very good performance, while C-ACT (AUC 0.769) showed good performance. Conclusion: All three questionnaires are effective for assessing current asthma control. Notably, ACT and ACQ-6 are superior instruments for accurately identifying patients at high risk for near-future exacerbations in an outpatient setting.

Introduction

Asthma in children is a chronic disease that poses a global public health challenge, affecting more than 262 million people worldwide and showing a steadily rising trend¹. For Thailand, the prevalence of asthma among children aged 6–14 years is approximately 12.50–14.60%, with the incidence of severe wheeze reported as high as 5.80–6.80%². Uncontrolled asthma has significant impacts on children, including limitations in physical activity and increased school absenteeism, which in turn adversely affect learning performance and overall quality of life²⁻⁴. Furthermore, there are important mental health consequences such as stress, anxiety, and depression resulting from social isolation^{5,6}, particularly among those with severe symptoms or frequent hospital admissions, who tend to have markedly reduced quality of life in all domains.

The primary goal of asthma management in children is to maintain a state of controlled asthma; therefore, assessment of the level of asthma control is a key component of care and treatment follow-up. The 2021 Thai pediatric asthma guideline⁷ has been revised to align with the Global Initiative for Asthma (GINA)⁸ recommendations and emphasizes the use of asthma control status as the basis for treatment adjustment. However, assessing asthma control in children remains challenging because of limitations in symptom communication, discrepancies in symptom perception between children and caregivers, limited involvement of children in treatment planning, and the diversity of triggering factors. Consequently, there is a need for assessment tools that are valid, reliable, and context-appropriate to efficiently monitor asthma control in children.

There are several asthma control questionnaires that are widely used, have been translated, and psychometrically tested in the Thai context, including the Childhood Asthma Control Test (C-ACT), the Asthma Control Test (ACT), and the Asthma Control Questionnaire (ACQ). C-ACT is designed for children aged 4–11 years and includes both a child-completed and a caregiver-completed section; it is considered a standard tool with good validity, reliability, and a positive correlation with FEV₁, although it has limitations related to interpretation complexity and variability of cutoff points across populations⁹. ACT is suitable for patients aged 12 years and older, with reports demonstrating high concordance with GINA-based standards and an association with FEV₁¹⁰. ACQ, applicable in children aged 6–16 years, is advantageous in its sensitivity to short-term changes in asthma control because it assesses symptoms over the preceding week, but some versions require spirometric measurements as part of the scoring¹¹.

Comparative studies of childhood asthma control questionnaires have shown that ACT and C-ACT demonstrate moderate to high agreement with GINA-base levels of asthma control^{10,12} and significant positive correlations with FEV₁ in both international and Thai settings, particularly among adolescents who can more accurately perceive and report their own symptoms^{10,13}. Evidence also suggests that ACQ, ACT, and C-ACT each correlate positively with FEV₁ and pediatric quality of life, although they differ in measurement properties and age coverage, underscoring the need for further evaluation across diverse clinical contexts¹⁴. However, in Thailand, direct comparative studies between the C-ACT, ACT, and ACQ within the same pediatric group are still lacking. This results in unclear information for selecting the most appropriate tool for Thai children. A major gap exists in monitoring patients over a wide age range (4–16 years), as the transition from C-ACT to ACT during adolescence causes data discontinuity. Although the ACQ covers this entire age range, data on its validity and reliability in Thai children remain limited.

According to statistics from Nakhon Pathom Hospital, hospitalization rates for asthma in children aged 1–15 years have shown a continuous upward trend between 2022 and 2024. A high proportion of these patients presented with exacerbations requiring treatment in the emergency department. Therefore, this study aims to analyze the agreement and compare the accuracy of the C-ACT, ACT, and ACQ-6 questionnaires in classifying asthma control against GINA guidelines and lung function (FEV). It also evaluates the predictive validity of these three tools for asthma exacerbations within a 3-month period. The findings are expected to help identify appropriate tools for monitoring asthma control, reduce the frequency of lung function tests, decrease the risk of exacerbations, and support proactive treatment planning to improve the quality of life for pediatric patients.

Materials and Methods

The study population consisted of pediatric patients with asthma aged 7–15 years and their caregivers. Participants were recruited from the Allergy and Immunology Clinic at the Outpatient Department of Nakhon Pathom Hospital, where they had been registered for treatment. The study was conducted between July 2025 and November 30, 2025, based on the following criteria:

Inclusion Criteria: 1) Aged between 7 and 15 years. 2) Diagnosed with asthma according to GINA guidelines. 3) History of continuous treatment for at least six months. 4) Ability to perform spirometry correctly. 5) Accompanied by a caregiver at every visit. 6) both patient and caregiver are able to read and understand the Thai language.

Exclusion Criteria: 1) Presence of other chronic lung diseases, including bronchopulmonary dysplasia, bronchiectasis, and cystic fibrosis. 2) Congenital heart disease affecting respiratory function or lung capacity. 3) Intellectual or developmental disabilities that may interfere with the understanding or completion of assessment questionnaires. 4) History of pneumonia within four weeks prior to study enroll.

The sample size was determined using the formula for agreement studies as follows:

$$n = \frac{[(Z_{1-\alpha/2})^2 \times P(1-P)]}{d^2}$$

Where:

$Z_{1-\alpha/2} = 1.96$ (at a 95% confidence level), $p = 0.70$ (expected agreement), $d = 0.10$ (allowable error for clinical assessment tools).

By substituting these values into the formula: $n = (1.96^2 \times 0.70 \times 0.30) / 0.10^2 = 81$ participants. The study planned to include two age groups—7–11 years and 12–15 years—with 81 participants per group, totaling 162 participants. To account for a potential 15% drop-out rate, the total sample size was increased to 186 participants. This total was divided into:

1) Age 7–11 years ($n=93$): Assessed using the C-ACT and ACQ-6

2) Age 12–15 years ($n=93$): Assessed using the ACT and ACQ-6

A total of 186 caregivers of children with asthma were also included. Participants were selected using purposive sampling according to the inclusion criteria.

Data Collection Instruments: the instruments used for data collection in this study included: 1) Demographic and Clinical Data Form: This form recorded baseline information, asthma history, comorbidities, and family history. 2) Current Treatment Records: Documentation of the patients' ongoing asthma medications. 3) Asthma Control Assessment Tools included the GINA, the C-ACT, the ACT, and the ACQ-6. 5) Clinical Records included spirometry results and incidences of asthma exacerbations.

Study Procedures: this prospective cohort study was conducted in two phases: baseline assessment and a 3-month follow-up. The procedures were as follows:

1) Age-Based Instrument Classification: Participants were categorized by age to receive appropriate assessment tools. The 7–11 years group was assessed using C-ACT and ACQ-6, while the 12–15 years group was assessed using ACT and ACQ-6.

2) Data Collection and Monitoring: Registered nurses and the principal investigator collected baseline data and ensured the completeness of all questionnaires (C-ACT, ACT, and ACQ-6) during both phases. They also recorded the incidence of asthma exacerbations occurring over the 3-month study period.

3) Blinded Clinical Assessment: To ensure independence and accuracy of the comparison, a specialist physician (acting as a research assistant) performed blinded assessments. This

involved evaluating asthma control levels according to GINA (2021) guidelines, conducting physical examinations, and performing spirometry, blinded to the questionnaire scores.

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp., Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$. The analysis methods were as follows:

1) Descriptive statistics were used to summarize demographic data, clinical characteristics, and assessment scores. Continuous variables are presented as mean, standard deviation (SD), median, and interquartile range (IQR). Categorical variables are reported as frequencies and percentages.

2) Analytical Statistics as follows:

- Cohen's kappa coefficient (kappa) and percent agreement were used to analyze the agreement, Pearson's correlation coefficient was used to analyze the relationships, and classification accuracy was evaluated using receiver operating characteristic (ROC) curve.

- The predictive ability of the tools was analyzed using logistic regression analysis and ROC curve analysis.

Results

Baseline Characteristics: A total of 186 children were enrolled with 93 participants in each age group. The younger group (7–11 years) had a mean age of 8.97 years; 56.99% were male, and the mean body mass index (BMI) was 17.96 kg/m², while the older group (12–15 years) averaged 13.67 years (51.61% female, mean BMI 20.92 kg/m²). Both groups were diagnosed at a similar mean age (~3.80 years), with asthma duration averaging 5.17 and 9.81 years, respectively.

Regarding healthcare utilization, the 7–11 years group had more ED visits (67.74% vs. 54.84%) but lower hospitalization rates than the older group (18.28% vs. 31.18%). Allergic rhinitis was the most common comorbidity (73.21% vs. 67.41%). While family history was prevalent in both (60–67%), household smoking exposure was higher in the 12–15 years group (17.20% vs. 7.53%).

For treatment, the younger group more frequently used ICS combined with SABA (68.82% vs. 50.54%). Conversely, the older group had a higher proportion of patients not using ICS (13.98% vs. 3.22%).

Validity and agreement of asthma control tools

Regarding the validity and agreement of the instruments, all three assessment tools demonstrated significant agreement with the GINA guidelines and significant correlations with lung function (FEV1). The ACT exhibited the highest performance, showing good agreement with GINA criteria and a very strong positive correlation with physiological parameters. In contrast, both the ACQ-6 and C-ACT achieved moderate agreement with the standard guidelines. While the ACQ-6 maintained a strong correlation with FEV1, the C-ACT showed the weakest relationship with both clinical guidelines and spirometric values (Table 1).

Table 1 Agreement with GINA guidelines and correlation with lung function (FEV1)

Predictors	Weighted kappa (K)	95% CI	Percent agreement	Correlation with FEV1 (r)	p-value
C-ACT (N=93)	0.449	0.316-0.582	55.914	0.269	0.009
ACT (N=93)	0.723	0.627-0.819	75.268	0.920	<0.001
ACQ-6 (N=186)	0.539	0.443-0.636	65.591	-0.688	<0.001

*C-ACT, Childhood Asthma Control Test; ACT, Asthma Control Test; ACQ-6, Asthma Control Questionnaire-6; CI, confidence interval.

Diagnostic accuracy for asthma control classification

The diagnostic performance of the three asthma control questionnaires in identifying uncontrolled asthma according to GINA guidelines is visually illustrated in Figure 1 and statistically confirmed as presented in Table 2. All three instruments demonstrated significant discriminative ability ($p < 0.001$ for all), with ACT showing the highest accuracy

(AUC=0.901, 95% CI: 0.837–0.965), followed by ACQ-6 with very good accuracy (AUC=0.830, 95% CI: 0.759–0.900) and C-ACT with good accuracy (AUC=0.774, 95% CI: 0.675–0.873). At their respective optimal cut-off points (C-ACT ≤ 22, ACT ≤ 19, ACQ-6 ≥ 1.0), the corresponding sensitivities, specificities, positive predictive values, and negative predictive values are summarized in Table 2.

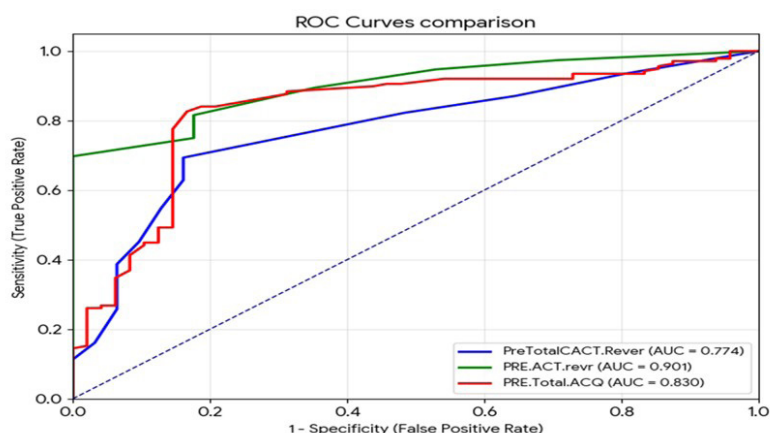


Figure 1 Receiver operating characteristic (ROC) curves comparing the diagnostic performance of C-ACT, ACT, and ACQ-6 for classifying asthma control according to GINA guidelines.

Table 2 Area Under the Curve (AUC) for the asthma control questionnaire according to GINA guidelines

Predictors	AUC	95% CI	Cut-point	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
C-ACT	0.774*	0.675-0.873	≤ 22	69.40	83.90	89.58	57.78
ACT	0.901*	0.837-0.965	≤ 19	69.70	100.00	100.00	62.00
ACQ-6	0.830*	0.759-0.900	≥ 1	84.10	79.20	89.00	71.35

* $p < 0.001$. AUC, area under the ROC curve; PPV, Positive Predictive Value; NPV, Negative Predictive Value

Predictive Validity for Asthma Exacerbations

The predictive validity of the assessment tools for asthma exacerbations over a 3-month follow-up period was evaluated using logistic regression and ROC curve analysis. In the adjusted model, patients classified as having uncontrolled asthma by ACT and ACQ-6 were at significantly higher risk of subsequent exacerbations, after adjustment for prior emergency department visits, hospitalizations,

and comorbid rhinitis (aOR 14.308; 95% CI 3.030–67.559 and 26.492; 95% CI 7.619–92.116, respectively). Conversely, uncontrolled status based on C-ACT did not reach statistical significance in the adjusted model ($p = 0.090$). ROC analysis further confirmed significant predictive accuracy for all tools ($p < 0.050$), with the ACT and ACQ-6 demonstrating very good accuracy (AUC = 0.879 and 0.872, respectively), while the C-ACT showed good accuracy (AUC = 0.769; Table 3)

Table 3 Logistic Regression Analysis and Predictive Validity of Baseline Questionnaire Scores for Predicting Asthma Exacerbations within a 3-Month Follow-up Period

Predictors	Adjusted OR (95% CI)	p -value	AUC (95% CI)	p -value
C-ACT*, (N=93)	3.236 (0.834-12.563)	0.090	0.769 (0.641-0.898)	0.004
ACT*, (N=93)	14.308 (3.030-67.559)	0.001	0.879 (0.797-0.961)	<0.001
ACQ-6*, (N=186)	26.492 (7.619-92.116)	<0.001	0.872 (0.851-0.929)	<0.001

*Uncontrolled vs Controlled / Partly. OR, odds ratio.

Discussion

Regarding agreement with the reference standard, all three questionnaires showed statistically significant concordance with the GINA criteria. ACT demonstrated good agreement, whereas ACQ-6 and C-ACT showed moderate agreement, reflecting the influence of developmental maturity and self-perception in adolescents aged 12–15 years, who are better able to observe and communicate clinical symptoms accurately and concretely. In this age group, the longer mean duration of asthma implies greater

experience living with the disease, which likely enhances their understanding of symptom patterns and bodily changes over time.

In contrast, the C-ACT and ACQ-6 may reflect the limitations of assessments in younger children, which rely on parental perception. Additionally, the ACQ-6 focuses specifically on symptom dimensions and medication use over a short 1-week recall period. This difference in timeframes directly impacts the classification of asthma control, particularly in patients with fluctuating symptoms.

Such patients might exhibit good control during the assessment week but remain uncontrolled according to standard guidelines when viewed in a broader monthly context, leading to lower levels of agreement compared to instruments with longer recall periods.

Furthermore, regarding the relationship with lung function parameters (FEV1), this study suggests that the ACT and ACQ-6 have a greater potential to reflect physiological lung function than the C-ACT. These findings indicate that adolescent maturity, duration of the disease, and the specialized clinic setting may be key factors contributing to the prominent relationship observed between the ACT/ACQ-6 and lung function in this study sample. This highlights that the selection of asthma control assessment tools should carefully consider both the patient's age and their ability for self-perception.

These findings are consistent with the study by Sultan et al.¹⁰, which reported a high level of agreement (92.90%) between ACT scores and GINA-defined asthma control levels in adolescents and adults. Additionally, the ACT showed a positive correlation with FEV1, where poorly controlled patients typically exhibited both low ACT scores and a concurrent decrease in FEV1. Akan et al.¹² compared ACT/pACT scores with GINA criteria and reported a moderate level of agreement (kappa 0.47), reflecting GINA-defined control levels. Furthermore, a systematic review¹³ evaluating the validity of the ACT and C-ACT in multiple countries indicated significant correlations with GINA-based classifications or physician assessments. These tools also demonstrated appropriate directional relationships with lung function indices, such as FEV1 and FEV1/FVC ratio, in children and adolescents. For Thai children, Sommanus et al.⁹ found that the Thai version of the C-ACT significantly and positively correlated with FEV1 over various follow-up periods; higher C-ACT scores were associated with both better GINA-defined control and higher FEV1 values. Collectively, these findings reflect a consistent trend where the ACT, C-ACT, and ACQ-6 not only demonstrate acceptable agreement with GINA standards but also correlate with lung function indicators in alignment with the underlying disease pathology.

In terms of diagnostic accuracy for classifying asthma control based on ROC-derived AUC revealed that all three assessment tools demonstrated statistically significant discriminative performance, ranging from good to excellent. Evaluation of diagnostic metrics highlighted distinct clinical strengths for each instrument. The ACQ-6 achieved the highest sensitivity at 84.10% with a cut-point of ≥ 1 , making it suitable as a proactive screening tool. Its responsiveness to short-term clinical changes facilitates the identification of uncontrolled patients who might otherwise be missed, thereby improving the efficiency of future exacerbation prevention. By contrast, ACT was distinguished by its very high specificity and positive predictive value, both reaching 100.00% at the cut-off score ≤ 19 , indicating a very high probability of truly uncontrolled asthma when the test is positive; this profile makes ACT more appropriate as a confirmatory tool to support decisions about stepping up treatment or revising control plans in adolescents. C-ACT, although showing a good AUC and relatively high specificity at the cut-off ≤ 22 , had only moderate sensitivity, suggesting a substantial risk of false-negative results in younger children. This aligns with the clinicophysiological dissociation phenomenon, in which clinical symptoms do not

always reflect the severity of underlying airway pathology, implying that reliance on C-ACT alone may underestimate risk and that decisions in young children should integrate caregiver observations and spirometric findings to ensure accurate and safe treatment adjustments.

The findings of this study are consistent with research in Thai children⁹, which indicated that the Thai version of the C-ACT demonstrated AUC values in the good to very good range relative to GINA control levels across various timeframes. This validates the C-ACT as a sufficiently accurate tool for differentiating between controlled and uncontrolled asthma in Thai clinical practice. In an international context¹⁵, both the ACT and C-ACT exhibited high AUC values compared to GINA guidelines. At a cut-point of 19, both instruments showed 100.00% specificity; however, the C-ACT demonstrated low sensitivity. This suggests that while both tools possess high potential for confirming uncontrolled asthma, cut-points must be carefully considered to avoid underestimating uncontrolled cases. Furthermore, Akan et al.¹² reported a good AUC for the ACT against GINA, with an optimal cut-point of approximately 21–22 for identifying uncontrolled asthma, which aligns closely with the score range utilized in the current study. Finally, a recent study¹⁶ comparing all three assessment tools against GINA criteria found a consistent pattern where the ACT achieved the highest AUC, followed by the C-ACT and ACQ in the good to very good range. That study also proposed new cut-points (C-ACT >22 and ACQ ≤ 0.930) to enhance sensitivity and specificity for optimal group classification.

The 3-month follow-up analyses showed that classification as uncontrolled asthma by any of the three questionnaires (C-ACT, ACT, and ACQ-6) was associated with an increased risk of subsequent exacerbations in a consistent direction. In particular, ACT and ACQ-6 remained significant predictors even after adjustment for key clinical covariates. However, the wide 95% confidence intervals observed for these tools suggest that the exact magnitude of risk should be interpreted with caution due to the limited number of events within the study period. This suggests that beyond their ability to classify current control status, these questionnaires possess predictive utility for near-future exacerbation events. When considered alongside the AUC results, which all ranged from good to very good, these findings support the application of all three tools for risk-stratified monitoring and treatment adjustment. Specifically, the ACQ-6 is effective as a proactive screening tool for identifying high-risk patients, while the ACT is suitable for adolescents to confirm and prioritize treatment step-up. For younger children, the C-ACT should be integrated with exacerbation history and parental observations to ensure a comprehensive risk assessment.

These findings are consistent with previous research in various settings. Meltzer et al.¹⁷ reported in a 12-week randomized controlled trial that increases in ACQ scores were associated with a 50.00% higher 2-week exacerbation risk, and that the composite ACQ score, integrating symptoms and reliever use, was a better predictor of future exacerbations than single-item indicator. Similarly, Gutierrez et al.¹⁸ reported that among patients in Allergy Clinics, uncontrolled ACT and ACQ scores were associated with higher rates of asthma exacerbations, emergency department visits, and hospitalizations during follow-up. Brand et al.¹⁹ also confirmed that composite asthma control scores, such

as the ACT, C-ACT, and ACQ, reflecting poor control are associated with reduced lung function and an increased risk of exacerbations, despite limitations such as short recall periods and the absence of direct exacerbation items within the scoring structure. Additionally, Ko et al.²⁰ demonstrated that pediatric patients with low ACT and C-ACT scores—specifically those falling within the uncontrolled range—spent more weeks in GINA-defined uncontrolled asthma than those with good control. This suggests that such low control scores can predict near-future loss of asthma control, which is directionally linked to a higher risk of exacerbations, even though these tools do not measure exacerbation events directly.

Study limitations include potential recall bias from self-reports and a brief follow-up missing seasonal variations. Low event rates resulted in wide 95% confidence intervals for ACT and ACQ-6, indicating limited statistical precision. Future multicenter, longitudinal research is warranted to enhance generalizability and provide more robust predictive estimates. To enhance the reliability and precision of the findings, future studies should consider increasing the sample size and extending the follow-up duration to at least one full year to better account for seasonal patterns. Furthermore, multicenter, longitudinal research is warranted to enhance generalizability and provide more robust predictive estimates.

Conclusions

The C-ACT, ACT, and ACQ-6 show high effectiveness for classifying asthma control and predicting 3-month exacerbation risk. ACQ-6 is suitable for proactive screening due to its high sensitivity, whereas ACT, with higher specificity, is useful for confirming treatment escalation in adolescents. C-ACT in younger children should be interpreted alongside clinical history and lung function to avoid underestimating risk, and selecting age-appropriate tools can enhance proactive asthma management and reduce hospitalizations.

Ethical statement

The study was approved by the Institutional Human Research Ethics Committee of Nakhon Pathom Hospital (protocol code COA No. 041/2025 NPH – REC No. 028/2025; approval date: July 7, 2025). Informed consent was obtained from all participants or their legal representatives.

Acknowledgements

The author would like to express sincere gratitude to the physician and nursing research assistants for their invaluable support throughout the study, as well as to the pediatric participants and their parents or caregivers for their generous cooperation and participation.

References

1. Network GA. The Global Asthma Report 2022. *Int J Tuberc Lung Dis* 2022;26(Suppl 1):1-104. doi:10.5588/ijtld.22.1010.
2. Network GA. Thailand – The Global Asthma Report 2022. Available from: <https://globalasthmareport.org/regions/thailand.php>.
3. Kouzegaran S, Samimi P, Ahanchian H, et al. Quality of life in children with asthma versus healthy children. *Open Access Maced J Med Sci* 2018;6(8):1413-8. doi:10.3889/oamjms.2018.287.
4. Battula M, Arunashekar P, Nagarajan VP. A prospective study to assess the quality of life in children with newly diagnosed asthma and their caregivers using the pediatric asthma quality of life questionnaire. *J Prim Care Community Health* 2020;11:2150132720961272. doi:10.1177/2150132720961272.
5. Khmour M, Abu Ghayyadeh M, Al-Hamed D, et al. Assessment of quality of life in asthmatic children and adolescents: A cross sectional study in West Bank, Palestine. *PLoS One* 2022;17(6):e0270680. doi:10.1371/journal.pone.0270680.
6. Aldirawi A, Al-Qudimat AR, Al Rawwad T, et al. Impact of asthma control on quality of life among palestinian children. *Sci Rep* 2025;15(1):7007. doi:10.1038/s41598-025-91756-9.
7. The Committee for the Development of Guidelines for the Treatment and Prevention of Asthma in Thailand for Pediatric Patients. Guidelines for the diagnosis and treatment of asthma in Thailand for pediatric patients (complete edition), 2021. Bangkok, Thailand: The Royal College of Pediatricians of Thailand and Pediatric Society of Thailand; 2021.
8. Asthma Gf. 2025 GINA Strategy Report: Global Strategy for Asthma Management and Prevention. Available from: <https://ginasthma.org/2025-gina-strategy-report/>.
9. Sommanus S, Direkwattanachai C, Lawpoolsri S, et al. Accuracy of childhood asthma control test among Thai childhood asthma patients. *Asian Pac J Allergy Immunol* 2018;36(3):152-8. doi:10.12932/ap-300517-0094.
10. Sultan KM, Al-Obaidy MW, Jaber AH. Comparison of Asthma Control Test (ACT) with (GINA) guidelines in the assessment of asthma control and determine if can use Asthma Control Test ACT as alternative to GINA guidelines in control asthma. *J Fac Med Baghdad* 2016;58(1):1-7. doi:10.32007/jfacmedbaghdad.581184.
11. Bime C, Gerald JK, Wei CY, et al. Measurement characteristics of the childhood Asthma-Control Test and a shortened, child-only version. *NPJ Prim Care Respir Med* 2016;26:16075. doi:10.1038/npjpcrm.2016.75.
12. Akan A, Dibek Mısırlıoğlu E, Civelek E, et al. Determining the best tool comparable with global initiative for asthma criteria for assessing pediatric asthma control. *Pediatr Allergy Immunol Pulmonol* 2021;34(3):89-96. doi:10.1089/ped.2020.1334.
13. Chu F, Kappel N, Akel M, et al. Validity of the childhood asthma control test in diverse populations: A systematic review. *Pediatr Pulmonol* 2023;58(5):1322-36. doi:10.1002/ppul.26342.
14. Voorend-van Bergen S, Vaessen-Verberne AA, de Jongste JC, et al. Asthma control questionnaires in the management of asthma in children: A review. *Pediatr Pulmonol* 2015;50(2):202-8. doi:10.1002/ppul.23098.
15. Koolen BB, Pijnenburg MW, Brackel HJ, et al. Comparing Global Initiative for Asthma (GINA) criteria with the Childhood Asthma Control Test (C-ACT) and Asthma Control Test (ACT). *Eur Respir J* 2011;38(3):561-6. doi:10.1183/09031936.00173710.
16. Agarwal S, Kumar P, Singh K, et al. GINA Versus Childhood Asthma Control Test (CACT), Asthma Control Test (ACT), and Asthma Control Questionnaire (ACQ) to Determine Asthma Control in Children. *Pediatr Pulmonol* 2025;60(11):e71362. doi:10.1002/ppul.71362.

17. Meltzer EO, Busse WW, Wenzel SE, et al. Use of the Asthma Control Questionnaire to predict future risk of asthma exacerbation. *J Allergy Clin Immunol* 2011;127(1):167-72. doi:10.1016/j.jaci.2010.08.042.
18. Gutierrez FJA, Galvan MF, Gallardo JFM, et al. Predictive factors for moderate or severe exacerbations in asthma patients receiving outpatient care. *BMC Pulm Med* 2017;17(1):77. doi:10.1186/s12890-017-0422-6.
19. Brand PL, Makela MJ, Szeffler SJ, et al. Monitoring asthma in childhood: Symptoms, exacerbations and quality of life. *Eur Respir Rev* 2015;24(136):187-93. doi:10.1183/16000617.00003614.
20. Ko FW, Hui DS, Leung TF, et al. Evaluation of the asthma control test: A reliable determinant of disease stability and a predictor of future exacerbations. *Respirology* 2012;17(2):370-8. doi:10.1111/j.1440-1843.2011.02105.x.

ORIGINAL ARTICLE

Efficacy of Using Interactive Video Learning on Medical Students' Satisfaction, Knowledge, and Engagement in a Large-Group Family Medicine Orientation Session

Passorn Rotpenpian

Department of Family Medicine, Panyanantaphikkhu Chonprathan Medical Center, Srinakarinwirot University

KEYWORDS

interactive online video-based learning;
large group teaching; medical students;
family medicine course

CORRESPONDING AUTHOR

Passorn Rotpenpian

Department of Family Medicine,
Panyanantaphikkhu Chonprathan Medical
Center, Srinakarinwirot University
222 Village No.1, Tiwanon Road, Bang Talat
Sub-district, Pak Kret District, Nonthaburi
11120, Thailand
E-mail: passornr@gs.wu.ac.th

ABSTRACT

Learning strategies in educational settings have significantly evolved in recent years, with an increasing integration of diverse technologies and a shift towards learner-centred education. However, the role of the instructor remains crucial, especially when faced with limitations such as time constraints and large student cohorts. These factors can hinder the instructor's ability to maintain the attention and engagement of the diverse learners in the classes. This study aimed to evaluate the effectiveness of developing and implementing interactive video-based learning as a substitute for traditional lectures in the orientation session of a Family Medicine course, which covers the course overview, learning activities, and assessment methods. Due to challenges in managing large student cohorts, this tool was developed to accommodate learner diversity and mitigate associated issues. Instructional effectiveness was assessed in terms of student satisfaction, content comprehension, and engagement. This study was conducted as an educational research project utilizing an action research methodology. The population and sample consisted of 165 fourth-year medical students enrolled at the Faculty of Medicine, Srinakharinwirot University, during the 2025 academic year. The study participants were asked to complete a pre-test, engaged with an interactive online instructional video, and to subsequently undertake a post-test and online satisfaction survey. The data obtained were analyzed using statistics and document analysis techniques. Overall, 147 students completed the study requirements, accounting for 89.09% of the total cohort. The mean pre-test score for these participants assessing their content comprehension was 4.59 out of 5, while the mean post-test score was 4.43 out of 5. Statistical analysis revealed that there was no significant difference between the pre-test and post-test scores ($z=1.930$, $p=0.054$). The average satisfaction score for the interactive online video-based learning approach was 3.25 out of 4 (corresponding to an 81.25% satisfaction rate). In conclusion, the study indicates that interactive online video-based instruction in large classes enhances student satisfaction and engagement. Although no statistically significant differences were observed in learning achievement, the findings underscore the pedagogical value of this instructional innovation in the given context.

Received: October 7, 2025

Revised: April 30, 2026

Accepted: May 6, 2026

Introduction

The Family Medicine course is an undergraduate component of the Doctor of Medicine programme delivered at the Faculty of Medicine, Srinakharinwirot University, Thailand, which covers the provision of care to patients and their families within primary care settings. This course emphasizes holistic and comprehensive care, addressing the physical, mental, social, and spiritual aspects of health. Furthermore, it focuses on health promotion, disease prevention, treatment, and rehabilitation at the individual, family, and community levels¹.

In recent years, there has been a shift in learning design patterns due to advancements in technology and an increased emphasis on the student-centred model. The role

of the teacher is essential in designing and implementing diverse teaching strategies to facilitate deep learning and promote learners' self-development.

In Thailand, Section 22 of the National Education Act B.E. 2542 places an emphasis on integrating critical thinking skills, knowledge acquisition, knowledge creation, and problem-solving abilities in learning programmes and course design². Furthermore, Srinakharinwirot University's Strategy No. 1 prioritises leadership in education³ by focusing on the development of skills essential for learning, working, and pursuing careers in the 21st century. The Doctor of Medicine programme at Srinakharinwirot University is dedicated to equipping learners with the competencies required for success in the medical field in the 21st century.

In the 2023 academic year, the family medicine course at Srinakharinwirot University included large-group teaching sessions. A survey on the course highlighted concerns in a number of areas, including the extended session durations, lack of participation from some students, and an issue of late arrivals to class. Some students were unaware of specific course details and had to seek clarification directly from the instructor. In the following academic year, 2024, because of the course duration and the high number of registered students, the course director trialed online video-based learning materials with the aim to promote and support students' self-study at their convenience. A number of students expressed interest in using such materials and participated in the trial, which had a reported satisfaction score of 79.6% among the participating students. However, some students did not participate in the trial, were seemingly unaware of the learning materials, or failed to access them. Additionally, certain students found the content difficult to understand and subsequently had to seek clarification from the instructor.

Due to these challenges, it became difficult for the instructor to maintain all the students' attention throughout the class. Furthermore, it is important to consider that different learners have diverse learning styles, which must be accounted for. To address these aspects, the author of the present report applied the Universal Design for Learning (UDL) framework to support the design of an updated and more effective family medicine course for large-group learning. UDL is a research-based approach aimed at enhancing the effectiveness of learning for all students. It helps to improve learning outcomes by promoting inclusive teaching strategies and by adapting the learning environment to better meet the needs of diverse learners⁴.

Numerous previous studies, both in Thailand and internationally, have explored online video-based learning and interactive video learning. However, within the field of medical education, such studies remain limited, particularly in relation to family medicine courses.

As a result, the author posed the question of whether the development of such a learning innovation could enhance the effectiveness of instructional design for large-group teaching. The objective of this study was to evaluate the effectiveness of developing and implementing an interactive online video for use during the orientation session of a family medicine course. The evaluation focused on students' satisfaction, comprehension, and class participation. The orientation session provides an overview of course operations, activities, and assessment methods. However, inconsistent attendance has led to uneven understanding, repeated inquiries, and administrative challenges in large classes. Therefore, an interactive video-based learning tool was developed to replace traditional lectures, support diverse learners, and promote consistent understanding. The anticipated outcomes included enhanced learning management, the advancement of educational innovation, and a greater accommodation of diverse learning styles. This approach could be expected to contribute to the development of medical students' competencies for delivering care within primary care settings. Moreover, the integration of interactive learning media in large-group teaching may facilitate continuous and systematic quality improvements in the course delivery and outcomes.

Materials and Methods

The present research employed an action research methodology within the field of educational research, incorporating both quantitative and qualitative designs.

The Family Medicine Practice I course is a foundational component of family medicine within the clinical clerkship curriculum. The course duration is one week and includes lectures, practical sessions, self-directed learning, and a summative assessment on the final day.

The study was conducted in May 2025. The target population comprised 165 fourth-year medical students from the Faculty of Medicine, Srinakharinwirot University, enrolled in the 2025 academic year. A census approach was employed, and all eligible students were invited to participate in the study. The inclusion criterion was medical students officially registered in the Family Medicine Practice I course. The exclusion criteria were: (1) students enrolled in the course who did not engage in any of the course-related activities; and (2) students who participated in the activities but failed to complete the pre- and post-intervention questionnaires, the satisfaction survey, or did not complete the course in its entirety.

The instruments employed in the present study comprised tools that were utilized in learning management, including a detailed lesson plan and a 20-minute interactive online video. The data collection instruments were: (1) a five-item multiple-choice pre-test questionnaire designed to assess student comprehension of each topic, evaluating recall knowledge aligned with the learning outcomes of the session. The pretest reliability yielded a Cronbach's alpha of 0.34.; (2) a satisfaction survey adapted from Evaluation Form PC 003 of Srinakharinwirot University⁵, which included an open-ended question; and (3) a five-item multiple-choice post-test questionnaire assessing student comprehension of each topic, intended to evaluate both recall and applied knowledge. The post-test was a parallel form of the pre-test. The posttest reliability yielded a Cronbach's alpha of 0.286. These assessments were excluded from the course's summative evaluation. The tests were developed based on a table of specifications and aligned with the orientation session's learning objectives, aiming to assess students' overall understanding of course procedures, session activities, assessment methods, assignment deadlines, and key terminology, thereby supporting its content validity.

A 20-minute online interactive video was developed, consisting of two sections: (1) an overview of course organization, learning activities, assessment methods, and key course terminology; and (2) assignment submission schedules. The learning objectives of the session and the video content were aligned with the pre- and post-session assessment questions. Throughout the video, embedded questions were used to reinforce learning; incorrect responses required repetition until the correct answer was selected before proceeding to the next section.

Data collection for the present study was performed following ethical approval. Initially, the researcher provided detailed information about the study to potential participants via an information sheet. This document clarified that students who chose not to participate in the research could still engage with the session content through self-study, using the standard pre-class materials distributed to all students. Subsequently, a research assistant, acting as the consent facilitator, distributed a link containing detailed information about the study and access to the course's Moodle learning

management system, which was restricted to individuals formally enrolled in the course. Participation was entirely voluntary and conducted anonymously. Upon completion of the data collection phase, the principal investigator

undertook the collation and verification of all the data to ensure its accuracy and integrity. A flow chart for the present study is provided in Figure 1.

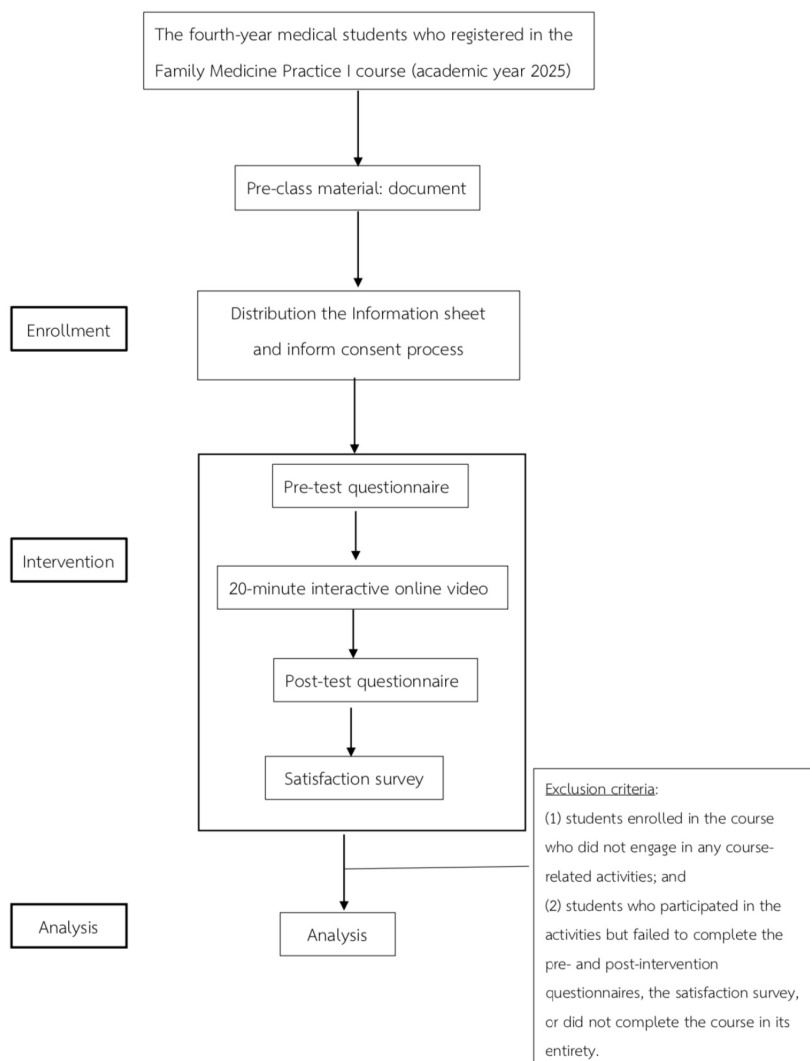


Figure 1 Flow of the study

Statistical analysis was undertaken using SPSS and Microsoft Excel software. Test of normality was performed using Kolmogorov-Smirnov test. Descriptive statistical analysis was performed to characterize the demographic data of the participants. Categorical data are reported as numbers and percentages, while continuous data are reported as the mean, standard deviation, median and inter-quartile range. Associations between the pre- and post-intervention measures were examined using the Wilcoxon Signed-Rank Test. Statistical significance was considered at a p -value less than 0.05, unless otherwise specified. Qualitative data were analyzed through document analysis.

Results

Characteristics of the participants

The total study population comprised 165 medical students. Among these, 158 participants (95.76%) fully engaged with the online activities; whereas 11 students participated in the activities but did not complete either the pre- and post-intervention questionnaires or the satisfaction survey and were thus excluded from the statistical analysis. Accordingly, the final cohort for the statistical analysis comprised 147 participants (89.09% of the overall study population). The participant flow in the study is presented in Figure 2. Demographic characteristics of the participants are presented in Table 1.

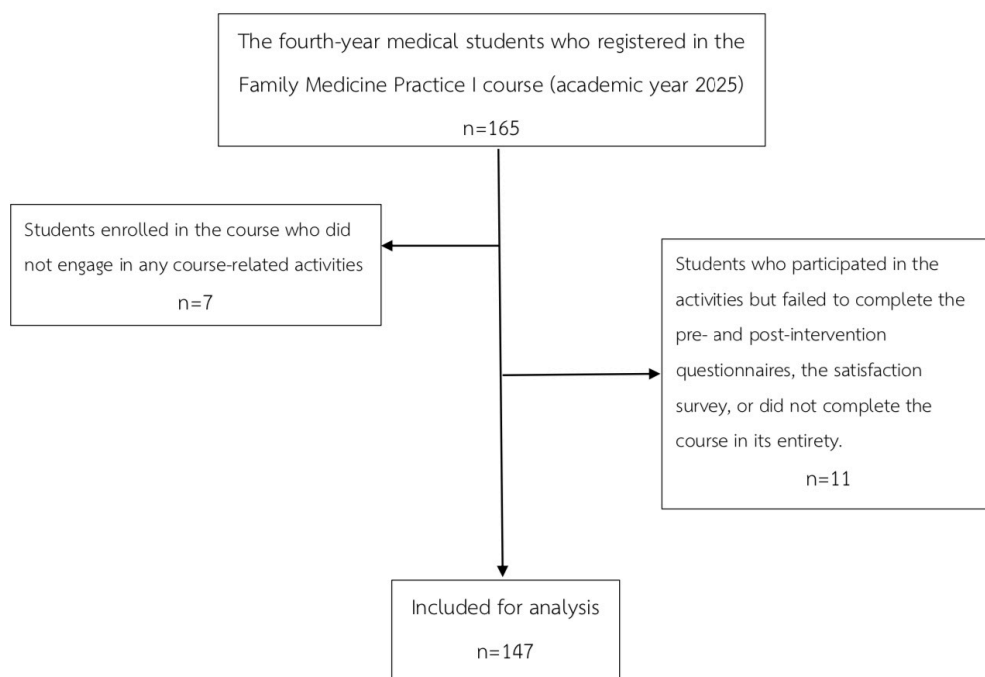


Figure 2 Flow diagram of participants

Table 1 Demographic characteristics of participants (n=147)

Baseline characteristics	n (%)
Gender	
Female	76
Male	71
Medical program	
Doctor of Medicine (M.D.); high school-entry program	127
Doctor of Medicine (M.D.); graduate-entry program	5
Doctor of Medicine Program (The Joint Medical Programme –Srinakharinwirot University and University of Nottingham, UK)	15

Pre- and post-test knowledge scores of the medical students who engaged with the programme's online video learning materials

The average total pre-test knowledge score of the medical students was 4.59 out of 5 points (91.8%), with a standard deviation of 0.78, a median of 5 and an interquartile range (IQR) of 1. The mean total post-test knowledge score was 4.43 out of 5 (88.6%), with a standard deviation of 0.75,

a median of 5, and an IQR of 1. Comparison of the pre- and post-test knowledge scores showed a difference between the scores, but this was not statistically significant ($z=-1.930$, $p=0.054$). Conversely, the item-based questions categorised by learning outcomes demonstrated a statistically significant difference ($p<0.05$). The assessment results obtained from the pre- and post-instruction testing are presented in Table 2.

Table 2 The assessment results of medical students obtained from pre- and post-instruction testing (n=147)

Assessment criteria aligned with the intended learning outcomes	Scores per test item (maximum of 1 point per item)						Z	p-value
	Pre-test			Post-test				
	Mean + -SD	Median	IQR	Mean + -SD	Median	IQR		
1. Instructional approaches and course content	0.86 + -0.35	1	0	0.99 + -0.75	1	0	+3.838	<0.001
2. Course practicum duration	0.99 + -0.12	1	0	0.93 + -0.12	1	0	-2.714	0.007
3. Assignment submission details	0.96 + -0.20	1	0	0.89 + -0.25	1	0	-2.668	0.008
4. Examination and assessment details	0.91 + -0.29	1	0	0.98 + -0.14	1	0	+2.324	0.020
5. Other aspects, such as attendance sign-in and procedures for addressing questions or concerns, are also included	0.87 + -0.34	1	0	0.65 + -0.48	1	1	-4.225	<0.001
Overall score	4.59 + -0.78	5	1	4.43 + -0.75	5	1	-1.930	0.054

Satisfaction scores of the medical students regarding the interactive online video learning

The average total satisfaction score of the medical students regarding the interactive online video learning was 3.25 out of 4 points (81.25%), with a standard deviation of 0.44. The highest-rated item was the clarity and detail of the explanations concerning the course objectives, outline, and assessment methods, which received a mean score of 3.72 (SD=0.47). This was followed by the effectiveness

of the instructional materials in facilitating students' understanding of the subject matter, with a mean score of 3.66 (SD=0.57). Subsequent items included the accessibility of the learning materials (mean=3.54, SD=0.74) and comprehension of the course's practical procedures following video-based instruction (mean=3.54, SD=0.62). The satisfaction scores of the medical students regarding the interactive online video learning are presented in Table 3.

Table 3 The satisfaction scores of medical students following participation in interactive online video learning

Topics	Mean (Maximum total score = 4)	SD
1. Level of interest in the lesson topic before watching the video	2.79	0.78
2. Engagement in self-guided supplementary learning before video viewing	2.58	0.90
3. Interaction and collaborative discussion on the lesson subject matter with senior students and peers	2.35	1.03
4. Perceived benefits	3.42	0.65
5. Accessibility of learning materials	3.54	0.74
6. Comprehension of the course's practical procedures following video-based instruction	3.54	0.62
7. Clear and detailed explanations of the course objectives, outline, and assessment methods are provided	3.72	0.47
8. The assignment of supplementary tasks to students is appropriate to the course and is accompanied by clear and detailed instructions	3.44	0.68
9. The course instructor provides channels for receiving student feedback and inquiries	3.47	0.72
10. The instructional materials facilitate students' comprehension of the subject matter	3.66	0.57
Overall average score	3.25	0.44

The qualitative feedback from the medical students, obtained after their participation in the interactive online video learning, was analysed using document analysis. The findings can be summarised as follows:

1. The content presented in the interactive video learning materials was considered to be clear, concise, and easily comprehensible. It effectively highlighted the essential knowledge needed, placing appropriate emphasis on core concepts deemed necessary for learners to master.

2. A key perceived benefit following participation was the enhancement of the students' time management skills. Learners reported an increased ability to engage in self-directed study via accessing the pre-class materials and interactive video content through the Moodle platform at times convenient to their individual schedules.

3. An area for improvement was the clarity of the course content. Some students expressed a desire to understand the assessment criteria for assignments in order to better prepare themselves.

Comments included: "...very well done. It saves students from having to wake up early for class. It gives students time to study on their own. And the video of the teacher repeating the lecture is clear and easy to understand...." (Case study No.1)

"...I like the interactive video format, which helps to emphasize points worth noting...." (Case study No.3)

Discussion

Overall, 158 medical students (95.76%) participated in the interactive online video learning, indicating a high level of student engagement with this instructional strategy. This finding supports the meaningful learning theory⁶, which emphasizes that effective teaching and learning should build upon learners' prior knowledge to facilitate their deeper understanding. Typically, the process begins with active engagement through the use of an advance organizer. In the present study, this was implemented by providing pre-class materials for students to skim through independently as part of their self-directed learning. Subsequently, students completed a pre-test self-assessment questionnaire, which helped them activate relevant prior knowledge from the pre-class activities. This prior knowledge was then integrated with new content presented in the interactive video, promoting systematic and deep learning. Moreover, this approach aligns with adult learning theory⁷, which emphasizes learners' ability to self-direct and identify their own learning needs in order to help them achieve the intended learning outcomes. In this study, the instructor provided easily accessible learning resources to support students' autonomous learning. Opportunities for self-assessment were incorporated, and the students were encouraged to consult the instructor as needed. This present study also supports the principles of Universal Design for Learning (UDL), which advocates for the creation of a flexible learning environment that accommodates the diverse needs of all learners⁴.

This approach is also consistent with the findings of Herbert et al.⁸, who investigated a blended learning model for large-group instruction involving 250 undergraduate students in an introductory pathology course. Their course was delivered through both on-site sessions and online components via Moodle. The study reported a high student attendance rate of 74%, indicating strong student engagement with the blended format. Similarly, Vakilian et al.⁹ examined the effectiveness of interactive virtual reality

videos compared to traditional online classroom teaching for teaching theoretical neurological stroke content during the COVID-19 pandemic. Their findings revealed that the use of interactive online videos significantly enhanced learner engagement, with students reporting feeling more involved in the learning process by them having to actively answer questions during the lessons. Furthermore, these findings aligned with those of Wang et al.¹⁰, who evaluated the impact of interactive video-based case study learning in clinical medical education. They concluded that interactive video learning significantly enhanced student engagement, extending beyond improvements in their test scores and learner satisfaction.

In the present study, the average total knowledge scores from both the pre- and post-tests were consistently above 80%, reflecting a high level of student achievement. Notably, the pre-test score averaged 91.8%, suggesting that the pre-class materials were effective in facilitating students' initial understanding of the content in this session. The use of an interactive online video further supported students' comprehension by addressing diverse learning styles and promoting deeper engagements with the subject matter. This was corroborated by the qualitative feedback, wherein the students indicated that the video format enhanced their grasp of key concepts and reinforced their knowledge of essential content.

Nevertheless, the post-test score average of 88.6% was less than the pre-test score, although statistical analysis indicated that this difference was not statistically significant. A more detailed item-by-item analysis revealed that the score levels were generally comparable. It was noteworthy that the majority of learners answered item 5 of the post-test incorrectly. This item pertained to the channels available for raising questions related to the course content. When examining the results of the post-instruction satisfaction survey, it was found that learners had a satisfaction level of 86.75% concerning the mechanisms for providing feedback or submitting questions. This indicates that the communication channels embedded within the system were at a satisfactory level. Nonetheless, it should be noted that the explanation regarding how to submit questions was presented verbally within the instructional video, but was not included in either the slide presentations or the distributed handouts. This constitutes an area for improvement in the subsequent academic year. Furthermore, when analyzing the individual test items categorized by specific learning objectives, all the items were found to exhibit statistically significant differences ($p < 0.05$).

This is in contrast to the finding in the study conducted by Cengiz GÜNDÜZALP¹¹, who investigated the use of interactive videos on educational websites for enhancing technological skills and digital literacy. That study concluded that the learners' post-instruction scores were significantly improved, particularly the learners utilizing the interactive video formats, who demonstrated higher levels of technological skills and digital knowledge. These findings suggest that interactive online video instruction could contribute positively to the development of 21st-century learning competencies.

In the present study, the overall average satisfaction score regarding interactive online video-based learning among the medical students was 81.25%, which was higher than that for the previous academic year, during which standard, non-interactive online videos that were accessible

via links were utilized. A satisfaction score of 79.6% was recorded during that academic year. This indicates that the instructional design utilizing interactive online videos via the LMS platform effectively enhanced learner satisfaction. These findings were consistent with the findings of Cristan Herbert et al.⁸, who investigated blended learning formats in large-group undergraduate medical education. In their study, 250 students enrolled in an introductory pathology course experienced both face-to-face and online instruction via the Moodle platform. The results revealed a high student satisfaction rate of 83.1% with the instruction. The learners seemed to value this instructional approach, particularly the inclusion of flexible and accessible interactive quizzes, which offered flexible access, enabling learners to engage with the content at any time and from any location, according to their individual schedules. This was further supported by the study performed by Jing Wang et al.¹⁰, which evaluated the impact of interactive video-based learning through a randomized controlled trial in clinical medical education. In that study, learners reported significantly greater satisfaction with the interactive videos compared to the standard video content. In the present study, one contributing factor to the increased satisfaction of the students may be the integration of a structured E-learning system combined with pre-learning assessments. These pre-tests allowed the students to self-evaluate their understanding prior to engaging with the pre-class materials. Upon identifying knowledge gaps, learners were more motivated to engage further with the content. This approach aligns with Gagné's theory of instructional design; specifically, the stage of stimulating the recall of prior learning. By activating prior knowledge, cognitive load can be reduced, and relevant information can be retrieved from long-term memory for use in working memory, thereby enhancing learning efficiency¹².

Furthermore, analysis of the itemized satisfaction responses revealed that Item 1, which assessed learners' interest in the lesson topic prior to engaging with the instructional video, received a satisfaction score of 69.75%, indicating a moderate level of satisfaction. Item 2, which measured the extent to which students engaged in independent study before viewing the video content, recorded a score of 64.5%, while Item 3, pertaining to learners' discussions of the lesson topic with senior students or peers, received the lowest score at 58.75%. These findings suggest that the preparatory phase prior to video-based learning may require further enhancement. For future instructional design, it is recommended that the sequencing of the instructional materials should be revised; for instance, distributing preparatory documents only after the completion of the pre-assessment. Such an approach may serve to foster greater intellectual curiosity and intrinsic motivation among learners, thereby promoting their more active engagement with pre-class learning materials.

Regarding the students' satisfaction scores for the content aspect of this instructional approach, it was found that the students rated very highly (over 90%) the clarity of the explanation of the course objectives, outlines, and the assessment and evaluation methods. Additionally, the use of instructional media was perceived to significantly enhance their understanding of the course content. In terms of the perceived benefits, the accessibility of the learning materials, understanding of the practical aspects after viewing the instructional videos, and the appropriateness of the assigned tasks received high satisfaction ratings, with scores

exceeding 80% in each case. These findings indicate that the use of interactive online video-based learning design could effectively support learners in both knowledge acquisition and through the ease of access to learning resources. These results are consistent with the findings of Rachel Ellaway and Ken Masters in their study of E-learning in Medical Education¹³, which highlighted that electronic learning systems (E-learning) in medical education can support instructional design across three key dimensions: content, functionality, and assessment. The present study employed Moodle as the learning management system (LMS), which fully supports these instructional design principles. It enables instructors to align content with learning objectives, utilize interactive video functionalities with embedded questions, and conduct pre- and post-learning assessments. Furthermore, the system provides learner progress tracking, allowing instructors to monitor individual participation, manage test access times, and analyze individual scores. The built-in feedback mechanism also allows instructors to collect question answers and receive feedback from learners efficiently and comprehensively.

Compared to the course design from the previous academic year, the instruction was originally designed as conventional lectures supplemented by links to online videos, which students could access at their convenience. This approach aimed to facilitate broader access to content for a diverse student population with various constraints and a large number of learners to improve performance outcomes. However, the instructors were unable to monitor individual student engagement in large classes, such as monitoring whether students attended or at what times they accessed the materials. Additionally, it was difficult to comprehensively track students' understanding of the content or gather feedback effectively. The development of an interactive online video learning system for large-class instruction via a Learning Management System (LMS) enabled addressing these limitations and effectively responding to these challenges. This aligns with the principles of online learning management, specifically the self-paced asynchronous learning model, where students independently determine their learning schedules without set times for interactions between learners and instructors. In this process, learners can complete assessments and watch videos at their convenience but are required to finish within the time frame specified by the instructor for course access¹⁴.

Furthermore, this approach corresponds with the ADDIE instructional design model, which involves the following steps: (1) Analysis of relevant components including content, learners, objectives, and environment; (2) Design of instruction, encompassing content structure, learning formats, course management systems, selection of supportive technologies, and assessment design; (3) Development and creation of the instructional system as designed; (4) Implementation of the instructional programme; and (5) Evaluation of learners, instructors, and administrative management in sequence^{15,16}.

This research encountered certain limitations, and a subset of the medical students did not fully participate in the pre- and post-intervention assessments or the satisfaction surveys, either by non-completion or partial completion. Despite these limitations, the study's findings provide valuable insights that can inform the ongoing development and enhancement of the instructional design and delivery of the course in future implementations.

Future research should consider evaluating the effectiveness of interactive online video instruction across a broader range of topics within the curriculum. Moreover, experimental studies could be undertaken to comparatively assess the effectiveness of this instructional approach relative to other online teaching modalities. Furthermore, qualitative research exploring learners' experiences and perceptions may yield further in-depth insights, which could inform the development of a more systematic and effective pedagogical framework.

Conclusions

The implementation of an interactive, online video-based instructional intervention has the potential to enhance the effectiveness of large-class teaching, particularly in terms of learner satisfaction and engagement. In this study, the mean satisfaction score among family medicine students was 81.25%, with a high participation rate of 95.76%, and a mean comprehension score of 88.6%. Although the overall knowledge outcomes were not statistically significant, the intervention effectively addressed learner engagement and course management objectives, indicating its value as an innovative instructional approach in this context.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of Panyanantaphikkhu Chonprathan Medical Center (protocol code EC 008/68; approval date: 16 May 2025).

Acknowledgements

The author wishes to express profound gratitude to the officers and secretary of the Division of Medical Education, Faculty of Medicine, Srinakharinwirot University, for their invaluable coordination and assistance in facilitating collaboration with the medical students. Sincere appreciation is also extended to the officer from the Department of Information Technology at Panyanantaphikkhu Chonprathan Medical Center for their expert guidance on the development and implementation of the Moodle SWU learning platform. The author is also deeply grateful to the medical students for their participation and contributions to this study.

References

1. Chitpitakert S, Hatthirai S, Thongwilai P, et al. Summary of work experience in family medicine. Nakorn Pathom: Community Health Systems Research and Development Office; 2010.
2. Dechakupt P, Yindeesuk P. Learning management in the 12th century. Bangkok: Chulalongkorn University Press; 2012.
3. Planning and Strategy Division, Srinakharinwirot University [Internet]. [cite 2025 March 25]. Available from: <http://planning.op.swu.ac.th/>.
4. Wolf T. Instructional design, engagement, and support (IDEAS): Universal design for learning (UDL) [Internet]. [cite 2025 August 17]. Available from: <https://www.umass.edu/ideas/universal-design-learning-udl>.
5. Quality Development Division Srinakharinwirot University. Quality assurance handbook. Bangkok: Division of General Affairs Office of The President Srinakharinwirot University; 2000.
6. Vargas-Hernandez JG, Vargas-Gonzalez OC. Strategies for meaningful learning in higher education. *J Res Instr* 2022;2(1):47-64. doi:10.30862/jri.v2i1.41.
7. Kaufman D, Mann KV. Teaching and learning in medical education: How theory can inform practice. 2013;11. doi:10.1002/9781118472361.ch2.
8. Herbert C, Velan GM, Pryor WM, et al. A model for the use of blended learning in large group teaching sessions. *BMC Med Educ* 2017;17(1):197. doi:10.1186/s12909-017-1057-2.
9. Vakilian A, Ranjbar EZ, Hassanipour M, et al. The effectiveness of virtual interactive video in comparison with online classroom in the stroke topic of theoretical neurology in COVID-19 pandemic. *J Educ Health Promot* 2022;11:219. doi:10.4103/jehp.jehp_1297_21.
10. Wang J, Jiang Y, Fu X, et al. Evaluating the impact of interactive video-based case-based learning in clinical medical education: A randomized controlled trial. *Front Med (Lausanne)* 2025;12:1556018. doi:10.3389/fmed.2025.1556018.
11. Gündüzalp C. Interactive videos in web-based education: Technology proficiency and digital literacy levels. *J Theor Educ Sci* 2024;17(3):738-64. doi: 10.30831/akueg.1390764.
12. Driscoll MP. Psychology of learning for instruction, 3rd ed. Chapter 10: Gagne's theory of instruction. Harlow: Pearson; 2005.
13. Ellaway R, Masters K. AMEE Guide 32: e-Learning in medical education Part 1: Learning, teaching and assessment. *Med Teach* 2008;30(5):455-73. doi:10.1080/01421590802108331.
14. Hrastinski A. Asynchronous and synchronous E-Learning. *EDUCAUSE Quarterly* 2008;4:51-55.
15. Aldoobie N. ADDIE model. *Am Int J Contemp Res* 2015;5(6):68-72.
16. Almomen RK, Kaufman D, Alotaibi H, et al. Applying the ADDIE—analysis, design, development, implementation and evaluation—Instructional design model to continuing professional development for primary care physicians in Saudi Arabia. *Int J Clin Med* 2016;7(8):538-46. doi:10.4236/ijcm.2016.78059.

ORIGINAL ARTICLE

Outcome of Nursing Service Process Improvement on Waiting Time for Coronary Angiography in Patients with Non-ST-Elevation Myocardial Infarction (NSTEMI)

Crisaporn Poonsong

Cardiac Catheterization Room, Trang Hospital

KEYWORDS

coronary angiography; non-ST-elevation myocardial infarction; waiting time

CORRESPONDING AUTHOR

Crisaporn Poonsong

Cardiac Catheterization Room, Trang Hospital
69 Kokkhun Road, Thap Thiang Sub-district,
Meung District, Trang 92000, Thailand
E-mail: crisaporn@gmail.com

ABSTRACT

Cardiac catheterization is currently the most widely used procedure for diagnosing and treatment of coronary artery disease, particularly coronary artery stenosis and occlusion. Stratifying the severity of patients with non-ST-segment elevation myocardial infarction (NSTEMI) is essential to guide guideline-directed management. However, prolonged waiting time for coronary angiography remains a major challenge in hospital settings, limiting timely access to recommended care. This quasi-experimental study employed a two-group post-test-only design to compare outcomes before and after implementation of a nursing service process model for NSTEMI patients undergoing coronary angiography. The model was implemented by integrating Lean principles with a seamless care system. A total of 54 participants were selected using purposive sampling, comprising 27 individuals in the control group and 27 in the intervention group. Data was extracted from medical records. A comparative analysis of the waiting time for coronary angiography and the incidence of major adverse cardiac events (MACE) during the waiting period were analyzed using the Mann-Whitney U test and Fisher's exact test, respectively, with a significance level set at 0.05. The findings revealed that most participants were male (64.8%), with a mean age of 62.7 ± 10.5 years. Following the implementation of the nursing service process model, the waiting time for coronary angiography was significantly reduced. The median waiting time decreased from 121 days (range 1-165; IQR=84) in the control group to 2 days (range 0-6; IQR=1) in the intervention group (Mann-Whitney U=22.00, $p<0.001$). The incidence of MACE during the waiting period showed a decreasing trend in the intervention group (7.4% vs. 11.1%), with a risk ratio (RR) of 0.67 (95% CI: 0.12-3.73). However, this difference did not reach statistical significance ($p=0.500$). Nevertheless, the findings suggest that the developed model may contribute to reducing waiting time for coronary angiography and demonstrates a trend toward a reduction in adverse cardiovascular events, although clinical outcomes did not reach statistical significance. This may be attributable to the limited sample size and the low number of observed events. The present study highlights the importance of patient flow management and healthcare service systems in improving the quality of care for patients with NSTEMI in a provincial hospital setting.

Received: December 24, 2025

Revised: August 6, 2026

Accepted: August 10, 2026

Introduction

Prolonged waiting time for coronary angiography in patients with non-ST-elevation myocardial infarction (NSTEMI) remains a major challenge for healthcare systems, particularly in resource-limited and developing country settings. Despite international guideline recommendations that very high-risk patients should undergo immediate invasive evaluation and high-risk patients within 24 hours, a substantial proportion of patients in real-world practice continue to experience delays of several weeks to months. This discrepancy is inconsistent with recommended standards of care and may adversely affect clinical

outcomes^{1,2}. Such delays primarily reflect system-level barriers rather than purely clinical factors. These include the absence of standardized risk stratification and prioritization processes, complex and fragmented appointment systems, the separation of outpatient and inpatient care pathways, and limited coordination across healthcare facilities and referral networks. As a result, patients with heterogeneous risk profiles are often managed within the same queue, leading to inappropriate prioritization and delayed access to invasive evaluation. Furthermore, although coronary angiography remains the standard diagnostic and therapeutic procedure for coronary artery disease, timely

access continues to be constrained by healthcare resource and service limitations^{1,4}. Current ESC and ACC/AHA/ACEP/NAEMSP/SCAI guidelines recommend that the timing of invasive management should be determined by patient risk rather than a uniform treatment strategy^{1,2}. Evidence from randomized trials, including TIMACS, VERDICT, RIDDLE-NSTEMI, and RAPID NSTEMI, suggests that the clinical effect of earlier invasive management varies according to patient risk and the timing of intervention, with potential benefits including reductions in recurrent or pre-procedural ischemic events⁵⁻⁹. However, despite strong evidence supporting risk-based invasive management, translating guideline recommendations into routine clinical practice remains challenging, particularly in healthcare systems where patient flow and referral pathways are fragmented.

Lean principles have been widely adopted to improve healthcare efficiency by eliminating non-value-added activities, reducing waiting time, and optimizing patient flow^{3,10-13}. Complementing this approach, seamless or integrated care emphasizes effective coordination across healthcare providers and service levels to ensure continuity of care throughout the patient journey^{14,15}. Together, Lean principles and integrated care provide a practical framework for redesigning referral pathways, strengthening care coordination, optimizing patient flow, and improving waiting-list management for patients with NSTEMI^{10,14,17}. However, despite growing evidence supporting Lean healthcare and integrated care, few studies have evaluated nursing service process models that combine risk-based prioritization, Lean workflow redesign, and seamless referral coordination to improve timely access to coronary angiography. Existing studies have mainly focused on treatment timelines or isolated process improvements within individual hospitals rather than integrated service redesign across referral networks.

Trang Hospital, a provincial cardiovascular referral center, prolonged waiting times for coronary angiography among patients with NSTEMI remain a major healthcare challenge despite ongoing efforts to improve care delivery. The previous care pathway relied primarily on outpatient specialist evaluation before scheduling coronary angiography, resulting in waiting times exceeding three months. In addition, fragmented communication between referring hospitals and the cardiac catheterization unit limited efficient referral coordination and queue management, contributing to hospital readmission (5%) and mortality (3%) during the waiting period¹⁶.

Accordingly, this study aimed to implement a nursing service process model for patients with NSTEMI undergoing coronary angiography by integrating Lean principles and seamless service concepts into routine care. The redesigned nursing service process strengthened risk-based prioritization, optimized waiting-list management, and improved referral coordination across the healthcare network. Its effectiveness was evaluated by comparing waiting time for coronary angiography and the incidence of major adverse cardiovascular events during the waiting period before and after implementation.

Materials and Methods

Study design

This study employed a quasi-experimental design with a historical control group and a two-group post-test-only approach to evaluate outcomes before and after the

implementation of a nursing service process model for patients with NSTEMI.

Participants

The target population comprised patients diagnosed with NSTEMI (ICD-10 I21.4) for whom the physician had developed a treatment plan for coronary angiography. Inclusion criteria included: (1) patients with a treatment plan for coronary angiography at Trang Hospital, and (2) patients aged 20 years and older. Exclusion criteria were: (1) patients who refused or did not consent to participate in the research, and (2) situations in which the physician altered or canceled the cardiac catheterization plan.

The sample size was calculated using Cohen's table based on an effect size of 0.5, a statistical power of 0.80, a significance level of 0.05, and a 95% confidence level. Purposive sampling was used to recruit 54 participants, comprising a control group of 27 patients who underwent coronary angiography between April and May 2024 and an intervention group of 27 patients who underwent the procedure between April and May 2025.

Intervention

The research intervention was a nursing service process model for patients with NSTEMI undergoing coronary angiography. The model was developed by integrating Lean principles, seamless service concepts, and international clinical practice guidelines, including the 2023 ESC guidelines for the management of acute coronary syndromes and the 2025 ACC/AHA/ACEP/NAEMSP/SCAI guidelines.

The model comprised three key components:

(1) Process analysis using Value Stream Mapping (VSM) and the DOWNTIME framework. The existing care process was mapped and analyzed using Value Stream Mapping (VSM), covering the entire continuum from diagnosis, referral, appointment scheduling, patient preparation, to the performance of coronary angiography. This analysis aimed to identify non-value-added activities and process bottlenecks. In parallel, waste was systematically evaluated using the DOWNTIME framework, including Defect, Overproduction, Waiting, Non-utilized talent, Transportation, Inventory, Motion, and Extra-processing. Key process issues identified included the absence of a risk stratification system, prolonged length of hospital stay, reliance on outpatient-based scheduling leading to delays, unclear nursing roles, multiple patient visits, and redundant procedural steps.

(2) A nursing service process pathway was developed, with nurses saving as the primary coordinators throughout the care continuum—from diagnosis to coronary angiography. The pathway incorporated risk stratification, interdisciplinary coordination, and continuity of care planning. Its primary objectives were to accelerate access to care, reduce delays, and enhance the efficiency of patient flow.

(3) The fast-track and seamless service system was developed by restructuring the conventional outpatient-based scheduling model into an in-hospitalization approach with a one-stop service for coronary angiography. In this redesigned model, real-time queue management was implemented to optimize patient flow and reduce delays, while a Hub-and-Spoke network coordination system was established between tertiary (hub) and referring (spoke)

hospitals through electronic communication platforms. This integrated system enabled advanced patient preparation prior to transfer and facilitated same-day invasive management during hospitalization, thereby enhancing continuity of care and improving timely access to coronary angiography.

Measurement

Data were collected using a general information record form, a medical history record form, a cardiac catheterization procedure record form, and a service process outcome record form. The primary outcome was waiting time for coronary angiography, defined as the interval from the physician's decision to perform coronary angiography until the date the procedure was completed. The secondary outcome was the occurrence of major adverse cardiovascular events (MACE) during the waiting period. MACE was defined as a four-component composite outcome including all-cause mortality, recurrent myocardial infarction, stroke,

and hospitalization for heart failure. Outcomes were assessed from the physician's decision to perform coronary angiography until the procedure date.

Statistical analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 25.0, with statistical significance set at $p < 0.05$. Descriptive statistics were used to summarize participant characteristics. Continuous variables were presented as median (interquartile range [IQR]) and categorical variables as frequency and percentage. Baseline characteristics were compared using the independent t-test, Mann-Whitney U test, Chi-square test, or one-sided Fisher's exact test, as appropriate. Between-group differences in coronary angiography waiting time were analyzed using the Mann-Whitney U test. The incidence of major adverse cardiovascular events (MACE) was compared using the one-sided Fisher's exact test.

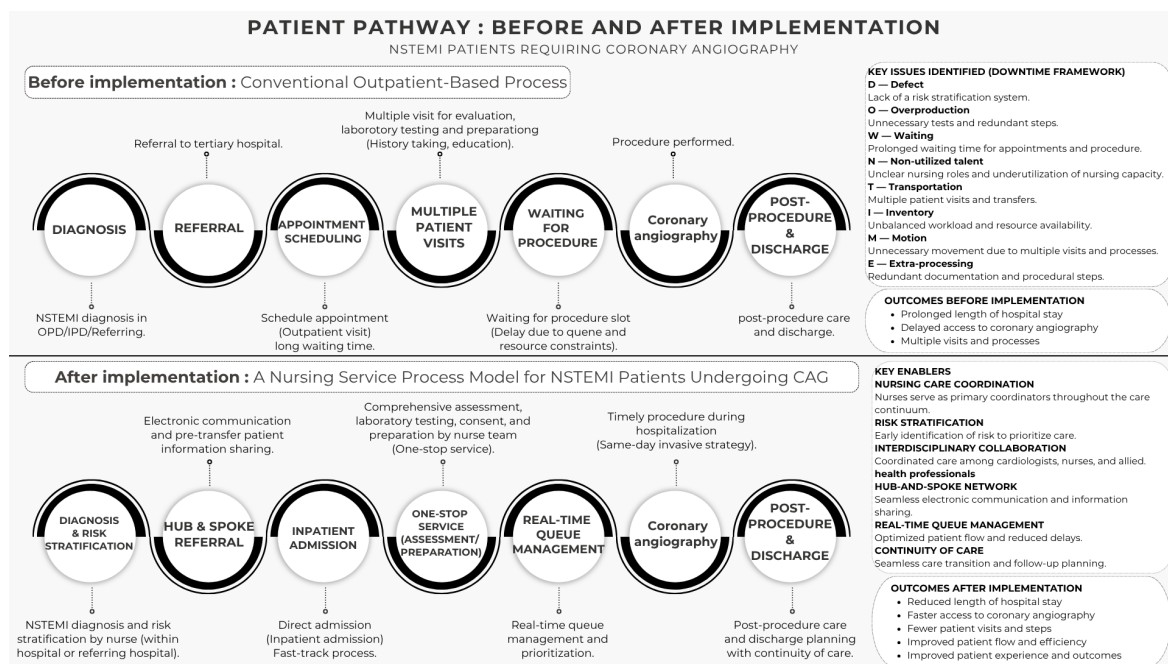


Figure 1 Patient pathway before and after implementation of the nursing service process model, demonstrating streamlined care, reduced waiting time, and improved coordination for NSTEMI patients undergoing coronary angiography. (created by author)

Results

A total of 54 participants were included. Male predominance was observed (64.8% vs. 35.2%), with a mean age of 62.7 ± 10.5 years. When comparing the baseline characteristics of the two patient groups, most variables were not significantly different between the intervention and control groups ($p > 0.05$), as shown in Table 1. However, dyslipidemia was significantly more prevalent in the control group compared to the intervention group ($p = 0.001$). In addition, adherence to clinical guidelines differed significantly between groups ($p < 0.001$).

Following implementation of the redesigned nursing service process, significant improvements in patient flow and timely access to coronary angiography were observed. The overall waiting time for coronary angiography ranged from 0 to 165 days (median 5.5 days). The Mann-Whitney U test demonstrated a statistically significant reduction in waiting time between groups (Mann-Whitney U=22.00, $p < 0.001$). Median waiting time decreased markedly from 121 days (range 1-165 days; IQR=84) in the pre-intervention group to 2 days (range 0-6 days; IQR=1) in the post-intervention group, corresponding to a median difference of 119 days,

as shown in Table 2. The incidence was lower in the intervention group compared with the control group (7.4% [2/27] vs. 11.1% [3/27]), with a risk ratio (RR) of 0.67 (95% CI: 0.12-3.73). However, this difference was not statistically significant (Fisher's exact test, one-sided $p=0.500$). Analysis of individual MACE components showed a similar pattern.

Overall, these findings suggest a potential trend toward reduced major adverse cardiovascular events during the waiting period in the intervention group; however, no statistically significant differences were observed. This may be attributable to the limited sample size and low number of events, as shown in Table 3.

Table 1 Comparisons of demographic and clinical characteristics between intervention and control groups

Characteristics	Total n=54 n (%)	Intervention group n=27 n (%)	Control group n=27 n (%)	p-value
Gender				0.776 ^a
Male	35 (64.8)	18 (66.7)	17 (63.0)	
Female	19 (35.2)	9 (33.3)	10 (37.0)	
Age (yr., mean±SD)	62.7±10.5	63.1±11.3	62.3±9.9	0.779 ^d
HT	38 (70.3)	18 (66.7)	20 (74.1)	0.551 ^a
DM	20 (37.0)	11 (40.7)	9 (33.3)	0.573 ^a
DLP	36 (66.7)	12 (44.4)	24 (88.9)	0.001 ^a
CVD	7 (13.0)	3 (11.1)	4 (14.8)	0.500 ^b
Smoking	19 (35.2)	10 (37.0)	9 (33.3)	0.776 ^a
Previous PCI	7 (13.0)	1 (3.7)	6 (22.2)	0.500 ^b
GRACE score, median (IQR)	134 (50)	134 (50)	132 (53)	0.986 ^c
Cardiogenic shock before procedure	2 (3.7)	1 (3.7)	1 (3.7)	0.500 ^b
Heart failure before procedure	13 (24.1)	9 (33.3)	4 (14.8)	0.101 ^b
Cardiac arrest before procedure	2 (3.7)	1 (3.7)	1 (3.7)	0.500 ^b
Risk level				0.098 ^b
Low	4 (7.4)	2 (7.4)	2 (7.4)	
Intermediate	25 (46.3)	15 (55.6)	10 (37.0)	
High	17 (31.5)	6 (22.2)	11 (40.7)	
Very high	6 (11.1)	4 (14.8)	2 (7.4)	
Coronary angiography*				0.096 ^b
Unobstructed	8 (15.0)	6 (23.1)	2 (7.7)	
Single vessel disease	12 (22.6)	7 (26.9)	5 (19.2)	
Multi vessel disease	32 (60.4)	13 (50.0)	19 (73.1)	
PCI Procedure*	6 (11.3)	3 (8.3)	3 (8.3)	0.500 ^b
Adherence to clinical guidelines				<0.001 ^b
Yes	27 (50.0)	26 (96.3)	1 (3.7)	
No	27 (50.0)	1 (3.7)	26 (96.3)	

*n=26 (dead=1), HT=Hypertension, DM=Diabetes Mellitus, DLP=Dyslipidemia, CVD=Cerebrovascular disease, PCI=Percutaneous Coronary Intervention, SVD=Single vessel disease, DVD=Double vessel disease, TVD=Triple vessel disease, a=Chi-square test (χ^2 test), b=one-sided Fisher's exact test, c=Mann-Whitney U test, d=independent t test

Table 2 Presents the changes in waiting time between intervention and control groups

Outcome	Total N=54 Median (IQR)	Intervention group N=27 Median (IQR)	Control group N=27 Median (IQR)	Mann-Whitney U test	p-value
Waiting time for CAG (day)	5.5 (119)	2 (1)	121 (84)	22.00	<0.001

Table 3 Presents the changes in MACE between intervention and control groups

Outcome	Total N=54	Intervention group N=27	Control group N=27	Risk ratio (95% CI)	p-value
MACE	5 (9.3%)	2 (7.4%)	3 (11.1%)	0.67 (0.12-3.73)	0.500
All-cause mortality	2 (3.7%)	1 (3.7%)	1 (3.7%)	1.00 (0.07-15.11)	0.500
Heart failure hospitalization	3 (5.6%)	1 (3.7%)	2 (7.4%)	0.50 (0.05-5.23)	0.500
Recurrent MI	0	0	0	NA	N/A
Stroke	0	0	0	NA	N/A

b=one-sided Fisher's exact test, NA=Not applicable, MI=myocardial infarction

Discussion

This study highlights the significant role of integrating Lean principles with seamless care coordination in improving the efficiency of healthcare service systems for patients with NSTEMI. In particular, the application of Lean principles in value stream management, waste reduction, and patient flow optimization represents a core mechanism of Lean healthcare. The findings of this study are consistent with existing evidence demonstrating that Lean principle and value stream mapping can effectively eliminate non-value-added steps, reduce waiting times, and enhance access to healthcare services^{11,17,18}. These results are further supported by studies conducted in the Thai healthcare context, which report similar improvements following Lean implementation^{3,12,13}. Systematic reviews of Lean healthcare have demonstrated that tools such as value stream mapping can identify non-value-added activities and facilitate process redesign, leading to significant reductions in waiting time and improvements in patient flow¹⁷. Furthermore, multiple systematic reviews have confirmed that Lean interventions positively impact patient flow, length of stay, and overall service efficiency, particularly in complex inpatient care settings^{11,18}.

In Thailand, Chaiyawaree et al.³ reported that the application of Lean principles in a cardiology clinic significantly reduced the number of service activities and waiting times. Similarly, Kamthongdee et al.¹² demonstrated that Lean-based outpatient service redesign reduced service points and average waiting times while improving patient satisfaction. In addition, Pakpises and Sitthiworanan¹³ reported that Lean implementation eliminated non-value-added process steps, reduced medication dispensing errors, shortened waiting time, and improved patient and staff satisfaction. Collectively, these findings suggest that Lean is not only an operational efficiency tool but also an effective approach for reducing waiting time and improving healthcare service processes.

From a clinical perspective, reducing waiting time for coronary angiography is crucial, as international guidelines from the 2023 ESC and the ACC/AHA/ACEP/NAEMSP/SCAI 2025 recommend that high-risk and very high-risk

NSTEMI patients undergo invasive management within 24 hours or sooner^{1,2}. However, in real-world healthcare settings, particularly in developing countries, access to such services is often delayed due to resource and system constraints. Therefore, the application of Lean principles in designing patient flow and coordination mechanisms plays a critical role in bridging the gap between guideline recommendations and real-world practice. Regarding clinical outcomes, although the incidence of MACE did not differ significantly between the two groups, a decreasing trend was observed following the intervention. This finding is consistent with evidence from meta-analyses indicating that an early invasive strategy may reduce recurrent ischemia, whereas consistent reductions in mortality and myocardial infarction have not been demonstrated across all patient populations^{5,19}. Randomized trials, including TIMACS, VERDICT, RIDDLE-NSTEMI, RAPID NSTEMI and the TAO trial, have reported heterogeneous findings regarding the optimal timing of invasive management. While very early or early invasive strategies have not consistently improved major clinical outcomes across all patients, they may reduce recurrent or pre-procedural ischemic events and provide greater benefit in selected higher-risk patients^{6-9,20}. Consistent with these findings, data from the nationwide SWEDEHEART registry²¹ demonstrated that coronary angiography performed within 24 hours, compared with 24-72 hours, was not associated with significantly lower mortality or major adverse cardiovascular events. These observations support the importance of healthcare systems that facilitate timely access through effective risk stratification and coordinated care pathways, rather than simply promoting earlier angiography for all patients. In this context, the Lean-based nursing service model evaluated in the present study may help bridge the gap between guideline recommendations and real-world practice by streamlining patient flow, reducing unnecessary process delays, and strengthening multidisciplinary care coordination.

Because this study employed a historical control design, changes observed over time may also reflect system-level or policy-related factors rather than the intervention alone. Therefore, the findings should be interpreted with caution.

Temporal confounders, such as changes in personnel, resources, or clinical practice, may have influenced the observed outcomes. Accordingly, these findings should be interpreted as associative rather than causal. Furthermore, baseline differences between the pre- and post-intervention groups, such as disease severity, presence of heart failure on admission, and prior PCI, may have influenced clinical outcomes. These potential confounding factors were not fully adjusted for in the analysis, which further limits causal interpretation. Importantly, the relatively small sample size, combined with the low event rate of secondary outcomes, suggests that this study may have been underpowered for secondary outcomes such as MACE. In addition, the estimation of effect sizes and confidence intervals may be subject to imprecision, which reduces the reliability of these estimates. Therefore, the results, particularly for secondary endpoints, should be interpreted with caution. This study was conducted in a single center and within a referral hospital context, which may limit the generalizability of the findings to other healthcare settings, particularly those with different resource availability and system structures.

Finally, although this study represents a pilot implementation demonstrating the potential of a Lean-based nursing service process model, scaling up to routine clinical practice requires careful consideration of system sustainability, particularly in terms of workload and manpower. Furthermore, the findings should be interpreted in light of the study's methodological limitations, including the relatively small sample size and single-center design, which may limit statistical power and external validity. Therefore, these findings should be regarded as hypothesis-generating rather than definitive evidence, and further validation in more rigorous research settings is warranted.

Building upon these preliminary findings, future research should include larger sample sizes to improve statistical power and extend to multicenter studies across diverse healthcare settings to enhance generalizability. In addition, prospective or time-series study designs would provide a more robust evaluation of the long-term sustainability, effectiveness, and scalability of this nursing service process model, thereby generating stronger evidence to support its broader implementation in routine clinical practice.

Conclusions

This study demonstrates that integrating Lean principles with seamless service concepts through the nursing service process model significantly improves service efficiency and reduces waiting times for coronary angiography among patients with NSTEMI. These findings underscore the importance of system-level interventions in optimizing patient flow and translating international guideline recommendations into real-world clinical practice. Although no statistically significant differences were observed in major adverse cardiovascular events, a favorable trend was noted, and the findings should therefore be interpreted as associative rather than causal. However, the results should be interpreted in the context of a quasi-experimental design with historical control, which may be influenced by time-related confounding factors and may limit causal inference. The proposed model appears to be particularly suitable for large secondary and tertiary care hospitals equipped with on-site cardiac catheterization facilities and well-established referral systems. It can be

effectively implemented through multidisciplinary clinical pathway development, structured queue management, and coordinated care across healthcare teams. At the policy level, these findings may support the strengthening of cardiovascular care networks to enhance service quality. Nevertheless, implementation should consider workload demands and workforce capacity to ensure long-term sustainability.

Ethical statement

This study was approved by the Human Research Ethics Committee of Trang Hospital (protocol code ID 019/04-2568; approval date: April 2, 2025). Informed consent was obtained from all participants or their legal representatives.

References

1. Byrne RA, Rossello X, Coughlan JJ, et al. 2023 ESC guidelines for the management of acute coronary syndromes. *Eur Heart J* 2023;44(38):3720-826. doi:10.1093/eurheartj/ehad191.
2. Rao SV, O'Donoghue ML, Ruel M, et al. 2025 ACC/AHA/ACEP/NAEMSP/SCAI guideline for the management of patients with acute coronary syndromes: A report of the American college of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation* 2025;151(13):e771-862. doi:10.1161/CIR.0000000000001309.
3. Chaiwaree N, Akkadechanunt T, Chitpakdee B. Application of lean concept to improve service processes in the cardiology clinic, Maharaj Nakorn Chiang Mai Hospital. *Thai Nurs Counc J* 2020;35(4):112-27. (in Thai)
4. Vrints C, Andreotti F, Koskinas KC, et al. 2024 ESC guidelines for the management of chronic coronary syndromes. *Eur Heart J* 2024;45(36):3415-537. doi:10.1093/eurheartj/ehae177.
5. Jobs A, Mehta SR, Montalescot G, et al. Optimal timing of an invasive strategy in patients with non-ST-elevation acute coronary syndrome: A meta-analysis of randomised trials. *Lancet* 2017;390(10096):737-46. doi:10.1016/S0140-6736(17)31490-3.
6. Mehta SR, Granger CB, Boden WE, et al. Early versus delayed invasive intervention in acute coronary syndromes. *N Engl J Med* 2009;360(21):2165-75. doi:10.1056/NEJMoa0807986.
7. Kofoed KE, Kelbæk H, Hansen PR, et al. Early versus standard care invasive examination and treatment of patients with non-ST-segment elevation acute coronary syndrome: VERDICT Randomized Controlled Trial. *Circulation*. 2018;138(24):2741-50. doi:10.1161/CIRCULATIONAHA.118.037152.
8. Milosevic A, Vasiljevic-Pokrajic Z, Milasinovic D, et al. Immediate versus delayed invasive intervention for Non-STEMI patients: The RIDDLE-NSTEMI Study. *JACC Cardiovasc Interv*. 2016;9(6):541-9. doi:10.1016/j.jcin.2015.11.018.
9. Kite TA, Ladwiniec A, Greenwood JP, et al. Very early invasive strategy in higher risk non-ST-elevation acute coronary syndrome: The RAPID NSTEMI trial. *Heart* 2024;110(7):500-7. doi:10.1136/heartjnl-2023-323044.
10. Poksinska B. The current state of Lean implementation in health care: Literature review. *Qual Manag Health Care* 2010;19(4):319-29. doi:10.1097/QMH.0b013e3181fa07bb.

11. Zepeda-Lugo C, Tlapa D, Baez-Lopez Y, et al. Assessing the impact of lean healthcare on inpatient care: A systematic review. *Int J Environ Res Public Health* 2020;17(15):5609. doi:10.3390/ijerph17155609.
12. Kamthongdee S, Noenchat A, Prakham J. Development of service model for reduce waiting times in outpatient department Namon Hospital Kalasin Province. *J Environ Community Health* 2024;9(1):726-36. (in Thai)
13. Parkpises W, Sitthiworanan C. Implementation of lean in outpatient department at 1 the 68th Public Health Center at Saphansung. *Thai J Pharm Pract* 2019;11(1):18-31. (in Thai)
14. World Health Organization. Framework on integrated, people-centred health services: Report by the Secretariat. Sixty-ninth World Health Assembly, provisional agenda item 16.1. Geneva: World Health Organization; 2016. Report No.: A69/39.
15. World Health Organization. Continuity and coordination of care: A practice brief to support implementation of the WHO framework on integrated people-centred health services. Geneva: World Health Organization; 2018.
16. Cardiac Catheterization Laboratory, Trang Hospital. Statistical report of the Cardiac Catheterization Laboratory, 2024. Trang: Trang Hospital; 2024. Unpublished internal report. (in Thai)
17. Marin-Garcia JA, Vidal-Carreras PI, Garcia-Sabater JJ. The role of value stream mapping in healthcare services: A scoping review. *Int J Environ Res Public Health* 2021;18(3):951. doi:10.3390/ijerph18030951.
18. Tlapa D, Zepeda-Lugo CA, Tortorella G, et al. Effects of Lean Healthcare on patient flow: A systematic review. *Value Health* 2020;23(2):260-73. doi:10.1016/j.jval.2019.11.002.
19. Navarese EP, Gurbel PA, Andreotti F, et al. Optimal timing of coronary invasive strategy in non-ST-segment elevation acute coronary syndromes: A systematic review and meta-analysis. *Ann Intern Med* 2013;158(4):261-70. doi:10.7326/0003-4819-158-4-201302190-00006.
20. Deharo P, Ducrocq G, Bode C, et al. Timing of angiography and outcomes in high-risk patients with non-ST-segment-elevation myocardial infarction managed invasively: Insights from the TAO trial (Treatment of Acute Coronary Syndrome With Otamixaban). *Circulation* 2017;136(20):1895-907. doi:10.1161/CIRCULATIONAHA.117.029779.
21. Eggers KM, James SK, Jernberg T, et al. Timing of coronary angiography in patients with non-ST-elevation acute coronary syndrome: Long-term clinical outcomes from the nationwide SWEDEHEART registry. *EuroIntervention* 2022;18(7):582-9. doi:10.4244/EIJ-D-21-00982.

ORIGINAL ARTICLE

Diagnostic Utility of Bile Cytology for Cholangiocarcinoma: A Real-World Comparison of ERCP-Guided Brush Cytology and PTBD-Derived Bile Cytology

Panunat Muangnoi¹, Panitpong Maroongroge², Chanchai Charonpongsuntorn¹, Supagone Wangsubtawee¹, Asawin Sudcharoen¹, Passagorn Sangsawangchot¹

¹Department of Internal Medicine, Faculty of Medicine, Srinakharinwirot University

²Department of Radiology, Faculty of Medicine, Srinakharinwirot University

KEYWORDS

cholangiocarcinoma; bile cytology; ERCP; PTBD; diagnostic sensitivity

CORRESPONDING AUTHOR

Panunat Muangnoi

Department of Internal Medicine, Faculty of Medicine, Srinakharinwirot University
62 Village No.7, Rangsit-Nakhon Nayok Road, Ongkharak Sub-district, Ongkharak District, Nakhon Nayok 26120, Thailand
E-mail: panunat@g.swu.ac.th

Received: July 15, 2026

Revised: August 11, 2026

Accepted: August 13, 2026

ABSTRACT

Bile cytology is widely used for the evaluation of biliary strictures and suspected cholangiocarcinoma; however, its diagnostic yield remains limited, and evidence directly comparing different sampling techniques is scarce. This study aimed to evaluate the diagnostic performance of bile cytology and compare the sensitivity of samples obtained by endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic biliary drainage (PTBD). This study conducted a single-center retrospective study of patients with histologically confirmed cholangiocarcinoma who underwent biliary drainage with cytologic sampling between January 2010 and June 2020. Bile specimens were obtained by ERCP-guided brushing or PTBD-derived bile collection. Cytologic findings were categorized as positive, negative, inconclusive, unsatisfactory, infection, or inflammation, with only positive results considered indicative of malignancy. A total of 66 patients were included, with a mean age of 62.2 years, and 72.7% were male. Perihilar cholangiocarcinoma was the most common subtype (43.9%), followed by distal (33.3%) and intrahepatic cholangiocarcinoma (19.7%). Cytology was obtained via ERCP in 53 patients (80.3%) and PTBD in 13 (19.7%). The overall sensitivity of bile cytology was 12.1% (8/66). Sensitivity was 13.2% (7/53) for ERCP and 7.7% (1/13) for PTBD, with an absolute difference of 5.5% that was not statistically significant ($p=1.00$). Non-positive results accounted for 87.9% of specimens, including negative (42.4%), inconclusive (27.3%), and unsatisfactory (10.6%) findings. Bile cytology therefore demonstrated low sensitivity for detecting cholangiocarcinoma in routine clinical practice. Although ERCP-derived specimens showed numerically higher sensitivity than PTBD-derived specimens, no statistically significant difference was observed. Given the small PTBD sample size and low overall positivity rate, these findings should not be interpreted as evidence of equivalence between the two methods, and negative cytology should be interpreted cautiously when clinical suspicion for malignancy remains high.

Introduction

Cholangiocarcinoma (CCA) is an aggressive hepatobiliary malignancy and remains one of the most diagnostically challenging cancers of the biliary tract. It represents a major health burden in several regions of Asia, particularly Southeast Asia, where incidence is substantially higher than in Western countries and chronic biliary inflammation and liver fluke infestation are endemic¹⁻⁴. Despite advances in diagnostic imaging and multidisciplinary management, prognosis remains poor because many patients present with advanced-stage disease. Curative surgical resection is the only potentially definitive treatment, but only a minority of patients are eligible at diagnosis. Accurate and timely diagnosis is therefore

essential for appropriate therapeutic decision-making and improved clinical outcomes.

CCA arises along the biliary tree and is anatomically classified as intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA)^{5,6}. Although multidetector computed tomography, magnetic resonance imaging, and magnetic resonance cholangiopancreatography can define the level of biliary obstruction, vascular involvement, and regional tumor extent, radiologic findings alone may not reliably distinguish malignant strictures from benign inflammatory or fibrotic conditions^{2,3}. This is particularly relevant in patients with indeterminate biliary strictures, in whom clinical, laboratory, and radiologic features may overlap. Obstructive jaundice and cholestatic liver enzyme abnormalities can occur in CCA

as well as benign inflammatory strictures, primary sclerosing cholangitis, IgG4-related cholangitis, and postoperative strictures. Histopathological confirmation therefore remains the gold standard⁷ and is often sought before major interventions such as surgery, systemic therapy, or locoregional treatment. However, adequate tissue acquisition can be difficult because of lesion location, fibrotic tumor composition, infiltrative growth, and limited cellularity^{7,8}.

Reliable tissue acquisition has become increasingly important in the era of precision oncology. Beyond confirming malignancy, pathological specimens may enable molecular profiling and biomarker-directed therapy, including assessment of potentially actionable alterations such as FGFR2 fusions, IDH1 mutations, HER2 amplification, and other targetable abnormalities. Thus, diagnostic material may influence both treatment selection and prognostic assessment. Nevertheless, advanced molecular testing is not universally available, particularly in resource-limited settings, and conventional cytological techniques remain an important component of the diagnostic pathway. Several approaches have been developed to improve tissue acquisition, including brush cytology, forceps biopsy, endoscopic ultrasound-guided fine-needle aspiration or biopsy (EUS-FNA/FNB), cholangioscopy-guided biopsy, fluorescence in situ hybridization (FISH), and molecular-based techniques. Many require specialized expertise, equipment, and additional healthcare resources. Bile cytology therefore remains widely used because it can be obtained during routine biliary drainage with minimal additional procedural risk.

Endoscopic retrograde cholangiopancreatography (ERCP) provides both biliary drainage, usually through stent placement, and an opportunity for brush cytology. However, the diagnostic performance of bile cytology remains suboptimal, with reported sensitivities of approximately 20% to 60%^{5,8}. This limited sensitivity has been attributed to the desmoplastic and infiltrative biology of CCA, low cellular exfoliation, and technical limitations in specimen collection and processing^{9,10}. These limitations are clinically important because delayed or inaccurate diagnosis may postpone appropriate oncologic treatment. ERCP is also technically complex, requires specialized equipment and experienced endoscopists, and may not be feasible in all patients^{11,12}.

Percutaneous transhepatic biliary drainage (PTBD) provides an alternative route for biliary decompression and sample acquisition. It can be performed by interventional radiologists and is particularly useful in patients with complex hilar obstruction, altered postoperative anatomy, failed ERCP, or anatomically inaccessible lesions^{13,14}. PTBD may also permit rapid, high-volume bile drainage and provides readily available bile specimens for cytological examination. Despite its widespread clinical use, PTBD-derived bile cytology is not routinely incorporated into standard diagnostic algorithms, and its diagnostic yield remains poorly defined^{15,16}.

Evidence regarding the real-world performance of bile cytology is limited. Previous studies have frequently included small or heterogeneous populations and have not always required histopathological confirmation^{17,18}, creating uncertainty regarding its true diagnostic performance in routine practice^{19,20}. Moreover, while ERCP-based brush cytology has been extensively studied, considerably less evidence is available for PTBD-derived bile specimens. Direct comparisons between ERCP- and PTBD-based cytology are

scarce, and whether the sampling route meaningfully affects diagnostic sensitivity remains unclear. This knowledge gap is particularly relevant in tertiary referral centers where PTBD plays an important role in managing biliary obstruction and where substantial volumes of bile may be available for cytological assessment.

Accordingly, this study aimed to evaluate the diagnostic performance of bile cytology in patients with histologically confirmed cholangiocarcinoma and to directly compare the sensitivity of ERCP- and PTBD-derived specimens, providing clinically relevant evidence to inform diagnostic strategies.

Materials and Methods

Study design and setting

This was a single-center retrospective study conducted at Princess Maha Chakri Sirindhorn Medical Center (MSMC), Faculty of Medicine, Srinakharinwirot University, Thailand. The study included consecutive patients treated between January 2010 and June 2020 who had histopathologically confirmed cholangiocarcinoma and underwent biliary drainage with cytologic sampling at our institution. Eligible patients were identified through retrospective review of hospital medical records. The study was designed to assess the real-world diagnostic performance of bile cytology among patients with a definitive histopathological diagnosis of cholangiocarcinoma.

Study population and reference standard

Patients were eligible for inclusion if they had histopathologically confirmed cholangiocarcinoma and had undergone biliary drainage with cytologic sampling during the study period. Histopathological confirmation served as the reference standard for malignancy. Patients without histopathological confirmation or without an available bile cytology result were not included in the analytic cohort. Because the study was designed to evaluate the sensitivity of bile cytology among patients with established cholangiocarcinoma, no benign biliary stricture control group was included. Accordingly, the analysis focused on cytological sensitivity and positivity rather than on the full spectrum of diagnostic accuracy measures. The anatomical subtype of cholangiocarcinoma and other clinical variables were obtained from contemporaneous medical records. Laboratory values were analyzed when available, and the number of patients contributing data to each laboratory parameter is reported in Table 2. This approach reflects the retrospective, real-world nature of the cohort and avoids assuming complete data availability for variables that were not measured uniformly during routine care.

Patient characteristics and data collection

Demographic, laboratory, procedural, and tumor-related data were retrospectively collected from the hospital medical records. Variables of interest included age, sex, method of biliary sampling, tumor size, and anatomical subtype of cholangiocarcinoma. Tumors were categorized as intrahepatic (iCCA), perihilar (pCCA), distal (dCCA), or other subtypes according to the diagnosis documented in the medical record.

Baseline laboratory parameters included total bilirubin, alkaline phosphatase (ALP), carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP), and carbohydrate antigen 19-9 (CA19-9), when available. Procedural information

included whether the cytologic specimen was obtained during endoscopic retrograde cholangiopancreatography (ERCP) or through percutaneous transhepatic biliary drainage (PTBD). As this was a retrospective study based on routine clinical practice, the availability of individual laboratory and tumor-related variables varied among patients.

Biliary drainage and cytology sampling

Bile cytology specimens were obtained using one of two sampling approaches: ERCP-guided brush cytology or PTBD-derived bile fluid collection. At our institution, ERCP was generally considered the first-line approach for biliary drainage and tissue acquisition when technically feasible. PTBD was used as an alternative approach in selected patients, particularly those with technically challenging anatomy, failed ERCP, or severe hilar obstruction.

ERCP provided access to the biliary tree for biliary decompression and simultaneous acquisition of cytologic specimens by brushing of the biliary lesion. In patients undergoing PTBD, bile fluid obtained through the percutaneous drainage route was collected for cytologic examination. The choice of drainage and sampling technique reflected routine clinical practice and was based on clinical and anatomical considerations.

Cytological processing and classification

All biliary cytology specimens were stained using the Papanicolaou method²³ and evaluated preoperatively by experienced hepatobiliary pathologists. Cytologic specimens were classified according to a five-tier system (classes I–V)^{16,21} based on established cytomorphologic criteria. These criteria included nuclear enlargement and irregularity, disorganized nuclear arrangement, abnormal chromatin patterns, irregular cluster margins, necrotic background, and variability in cluster size^{22,26}.

For clinical and analytical interpretation, cytologic findings were further categorized into six diagnostic groups: positive, negative, inconclusive, unsatisfactory, infection, and inflammation. For the primary analysis, only specimens classified as positive were considered cytologically diagnostic of malignancy, whereas negative, inconclusive, unsatisfactory, infection, and inflammation categories were grouped as non-positive^{24,25}. The classification system was applied in accordance with established cytopathological reporting standards^{27,28}.

Study outcomes and definitions

The primary outcome was the diagnostic sensitivity of bile cytology for detecting cholangiocarcinoma, assessed both in the overall study population and separately according to the sampling route. Sensitivity was defined as the proportion of patients with histopathologically confirmed cholangiocarcinoma who had positive bile cytology.

The principal comparison was the sensitivity of ERCP-derived versus PTBD-derived specimens. Secondary outcomes included the distribution of individual cytologic categories and the proportions of positive and non-positive specimens according to sampling technique. The absolute difference in sensitivity between the two sampling methods and the prevalence ratio for cytologic positivity were also evaluated.

Because the study population consisted exclusively of patients with histopathologically confirmed cholangiocarcinoma, the study was designed primarily to assess sensitivity and cytologic positivity. Specificity, positive predictive value, and negative predictive value could not be reliably estimated because patients without cholangiocarcinoma were not included in the analytic cohort.

The route of biliary drainage and cytology sampling was determined by routine clinical and anatomical considerations rather than by random assignment. Therefore, comparisons between ERCP- and PTBD-derived specimens were intended to describe differences in observed cytological yield rather than to establish causal superiority or statistical equivalence between the two procedures. In addition to overall sensitivity, the analysis examined the distribution of individual cytological categories because negative, inconclusive, and unsatisfactory specimens represent clinically distinct forms of diagnostic limitation. The proportion of non-positive results was therefore reported together with the absolute difference in sensitivity and the prevalence ratio for cytological positivity. Given the small number of positive results and the limited size of the PTBD group, inferential comparisons were interpreted cautiously.

Statistical analysis

Descriptive analyses were used to summarize demographic, laboratory, tumor-related, procedural, and cytologic characteristics of the study population. Cytologic findings were summarized for the overall cohort and stratified according to sampling method. The proportions of positive cytology results were calculated separately for ERCP- and PTBD-derived specimens, and the absolute difference in sensitivity between the two techniques was reported. The prevalence ratio was additionally calculated as an effect estimate for cytologic positivity.

Comparisons of categorical variables were performed using Fisher's exact test, as appropriate, given the limited number of positive cytology results and the relatively small number of patients in the PTBD group. Continuous variables were evaluated using the Wilcoxon rank-sum test when appropriate. All statistical tests were two-sided, and a p -value <0.05 was considered statistically significant. Statistical analyses were performed using R.

Results

A total of 66 patients with histologically confirmed cholangiocarcinoma were included in the study. The mean age was 62.2 years (range, 36–90 years), and most patients were male (72.7%, $n=48$), while females accounted for 27.3% ($n=18$). The mean tumor size was 4.29 ± 2.58 cm, with lesion sizes ranging from 0.7 to 10.9 cm. Regarding tumor location, perihilar cholangiocarcinoma was the most common subtype, accounting for 43.9% ($n=29$) of cases, followed by distal cholangiocarcinoma in 33.3% ($n=22$) and intrahepatic cholangiocarcinoma in 19.7% ($n=13$). Two patients (3.0%) were classified as having other tumor subtypes. These findings indicate that the study population was predominantly composed of patients with extrahepatic cholangiocarcinoma, particularly those with perihilar involvement (Table 1).

Bile cytology specimens were obtained via ERCP in 53 patients (80.3%) and PTBD in 13 patients (19.7%). Overall, positive cytology results were observed in 8 patients, yielding a sensitivity of 12.1%.

When stratified by sampling method, the sensitivity of bile cytology was 13.2% for ERCP and 7.7% for PTBD. The absolute difference in sensitivity between the two methods was 5.5%, with no statistically significant difference ($p=1.00$).

The distribution of cytology results was comparable between the two groups, with a high proportion of non-diagnostic outcomes, including negative, inconclusive, and unsatisfactory samples.

Table 1 Baseline and tumor characteristics of the included patients (n = 66)

Baseline characteristics	Overall (n = 66)
Age, mean (range), years	62.2 (36-90)
Sex, n (%)	
Male	48 (72.7)
Female	18 (27.3)
Tumor size characteristics	
Number of patients	66
Tumor size, mean $\pm$ SD (cm)	4.29 $\pm$ 2.58
Tumor size, range (cm)	0.7-10.9
Tumor subtype, n (%)	
Intrahepatic (iCCA)	13 (19.7)
Perihilar (pCCA)	29 (43.9)
Distal (dCCA)	22 (33.3)
Other ²⁷	2 (3.1)

Baseline laboratory parameters reflected advanced biliary obstruction in a substantial proportion of patients. The mean total bilirubin level was 16.28 ± 11.93 mg/dL, with values ranging from 0.27 to 59.19 mg/dL. Similarly, alkaline phosphatase (ALP) levels were markedly elevated, with a mean value of 445.44 ± 314.62 U/L (range, 75-1673 U/L). Tumor marker levels demonstrated considerable variability among patients. The mean carcinoembryonic antigen (CEA) level was 113.38 ± 611.22 ng/mL, ranging from 0.7 to 424.27 ng/mL. Alpha-fetoprotein (AFP) levels remained relatively low, with a mean value of 1.46 ± 1.10 ng/mL. In contrast, carbohydrate antigen 19-9 (CA19-9) levels were markedly elevated in many patients, with a mean value of $8,133.68 \pm 27,849.3$ U/mL and a range extending from 0.6 to more than

120,000 U/mL. The wide distribution of tumor marker values likely reflects the heterogeneity of tumor burden and disease stage within the study population (Table 2).

Among the 66 patients included in the study, bile cytology specimens were obtained predominantly through ERCP. A total of 53 patients (80.3%) underwent ERCP-guided sampling, whereas only 13 patients (19.7%) underwent PTBD-derived bile collection. This distribution reflects the routine clinical practice at our institution, where ERCP is generally considered the first-line approach for biliary drainage and tissue acquisition, while PTBD is reserved for selected patients with technically challenging anatomy, failed ERCP, or severe hilar obstruction.

Table 2 Baseline laboratory parameters

Parameter	n	Mean $\pm$ SD	Range
Total bilirubin (mg/dL)	66	16.28 ± 11.93	0.27-59.19
ALP (U/L)	66	445.44 ± 314.62	75-1,673
CEA (ng/mL)	49	113.38 ± 611.22	0.7-424.27
AFP (ng/mL)	33	1.46 ± 1.10	1.01-5.98
CA19-9 (U/mL)	53	$8,133.68 \pm 27,849.3$	0.6-120,000

The distribution of cytological diagnoses is shown in Table 3. Positive cytology results were identified in only 8 patients (12.1%), whereas negative cytology findings were reported in 28 patients (42.4%). Among the non-positive results, inconclusive cytology represented the second most frequent category, accounting for 18 cases (27.3%).

Unsatisfactory specimens were observed in 7 patients (10.6%), indicating inadequate cellular material for definitive interpretation. Inflammatory changes without evidence of malignancy were reported in 4 patients (6.1%), while evidence of infection was identified in one patient (1.5%).

When stratified according to sampling technique, positive cytology was observed in 7 of 53 ERCP-derived specimens (13.2%) and in 1 of 13 PTBD-derived specimens (7.7%). Negative cytology findings were reported in 43.4% and 38.5% of ERCP and PTBD specimens, respectively. Notably, inflammatory cytology findings were more frequently observed in PTBD-derived samples (30.8%) than in ERCP-derived samples, although the small number of PTBD cases limits definitive interpretation.

Overall, non-positive results constituted the vast majority of cytological diagnoses, accounting for 87.9% of

all specimens, highlighting the limited diagnostic sensitivity of bile cytology in routine clinical practice (Table 3 and Figure 1).

Baseline laboratory data were variably available because investigations were performed according to routine clinical indications. Total bilirubin and ALP values were available for all 66 patients, whereas CEA, AFP, and CA19-9 measurements were available in 49, 33, and 53 patients, respectively. This pattern is consistent with retrospective real-world data collection and should be considered when interpreting the descriptive laboratory findings.

Table 3 Distribution of bile cytology results according to sampling method

Result	ERCP (n=53)	PTBD (n=13)	Total (n=66), %
Positive	7 (13.2)	1 (7.7)	8 (12.1)
Negative	23 (43.4)	5 (38.5)	28 (42.4)
Inconclusive	16 (30.2)	2 (15.4)	18 (27.3)
Unsatisfactory	6 (11.3)	1 (7.7)	7 (10.6)
Inflammation	0 (0.0)	4 (30.8)	4 (6.1)
Infection	1 (1.9)	0 (0.0)	1 (1.5)

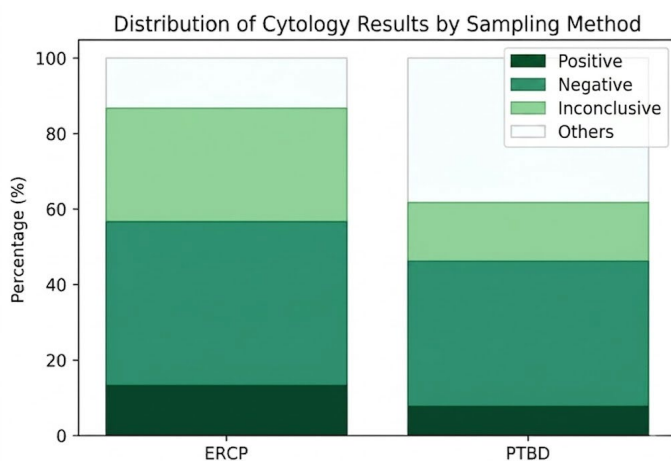


Figure 1 Distribution of bile cytology results stratified by sampling method.

The overall diagnostic sensitivity of bile cytology for cholangiocarcinoma was 12.1% (8/66). Among patients who underwent ERCP-guided sampling, the sensitivity was 13.2% (7/53), whereas the sensitivity among PTBD-derived specimens was 7.7% (1/13). The absolute difference in sensitivity between the two techniques was 5.5%, favoring ERCP. However, this difference did not reach statistical significance ($p=1.00$). The prevalence ratio for cytological positivity was 1.72 for ERCP compared with PTBD, although

the confidence interval was wide because of the limited sample size and low number of positive cases.

When dichotomized into positive and non-positive categories, 86.8% of ERCP-derived specimens and 92.3% of PTBD-derived specimens yielded non-positive results. These findings indicate that both sampling techniques exhibited similarly limited sensitivity for the detection of cholangiocarcinoma (Tables 4 and 5; Figure 2).

Table 4 Dichotomized cytology outcomes (positive vs. non-positive)

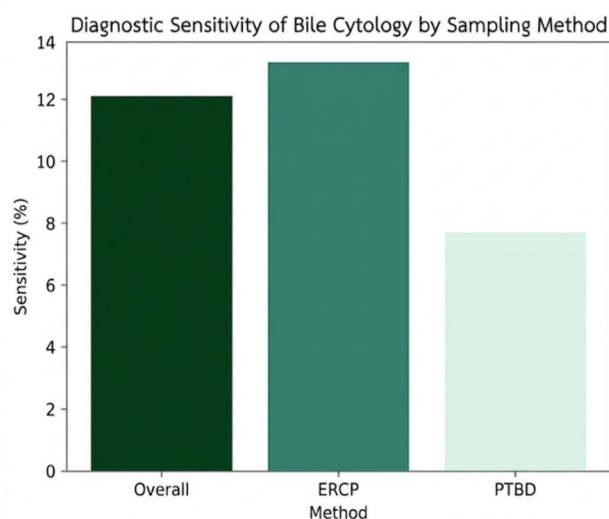
Method	Positive n (%)	Non-positive n (%)
ERCP	7 (13.2)	46 (86.8)
PTBD	1 (7.7)	12 (92.3)
Total	8 (12.1)	58 (87.9)

Regarding cytological adequacy, 25 of 66 specimens (37.9%) were classified as either inconclusive or unsatisfactory. These non-diagnostic categories accounted for 22 of 53 ERCP-derived specimens (41.5%) and 3 of 13 PTBD-derived specimens (23.1%). Given the small PTBD sample size, these proportions should be interpreted descriptively rather than as evidence of a difference between techniques. Among the 58 non-positive specimens, negative cytology accounted for 28 cases (48.3%), inconclusive findings for 18 (31.0%), and unsatisfactory specimens for

7 (12.1%); the remaining results reflected inflammation or infection. Thus, almost half of all non-positive specimens represented indeterminate or technically non-diagnostic categories rather than unequivocally negative cytology. This distinction is clinically relevant because the causes and implications of a negative specimen may differ from those of an inconclusive or inadequate specimen. Reporting the full distribution therefore provides a more informative description of real-world cytological performance than a binary positive-versus-negative classification alone.

Table 5 Positivity rate and diagnostic performance of bile cytology according to sampling method

Parameter	ERCP (n=53)	PTBD (n=13)	Overall (n=66)	Effect estimate
Positive cytology, n (%)	7 (13.2)	1 (7.7)	8 (12.1)	-
Non-positive, n (%)	46 (86.8)	12 (92.3)	58 (87.9)	-
Sensitivity (%)	13.2	7.7	12.1	
Absolute difference sensitivity (%)	-	-	5.5	ERCP vs PTBD
Prevalence ratio (95% CI)	1.72	-	-	ERCP vs PTBD
<i>p-value</i>	-	-	1.00	ERCP vs PTBD

**Figure 2** Diagnostic sensitivity of bile cytology by sampling method.

Although ERCP-derived specimens demonstrated a numerically higher sensitivity than PTBD-derived specimens, the absolute difference of 5.5% was small and did not reach statistical significance. The prevalence ratio of 1.72 suggested a trend toward greater diagnostic yield with ERCP; however, the wide confidence interval reflects substantial uncertainty arising from the limited sample size and the low number of positive cytological results. Consequently, the observed difference should be interpreted cautiously and may not represent a clinically meaningful advantage of one sampling technique over the other.

Notably, more than 85% of specimens obtained through either technique yielded non-positive results. This consistently high proportion of negative, inconclusive, or unsatisfactory findings underscores the limited standalone diagnostic value of bile cytology in patients with suspected cholangiocarcinoma. These data suggest that the route of sample acquisition may have less influence on diagnostic performance than the intrinsic biological characteristics of the tumor, including low cellular exfoliation and dense desmoplastic stromal architecture. Therefore, regardless of the drainage modality used, bile cytology should be interpreted within the broader clinical context and supplemented by additional diagnostic modalities whenever malignancy remains strongly suspected.

Discussion

In this retrospective study of patients with histologically confirmed cholangiocarcinoma, bile cytology demonstrated a low overall sensitivity of 12.1% for detecting malignancy. ERCP-derived specimens showed a numerically higher sensitivity than PTBD-derived specimens (13.2% vs. 7.7%); however, no statistically significant difference was detected between the two sampling approaches. In addition, more than 85% of specimens from either method yielded non-positive results, with a substantial proportion categorized as inconclusive or unsatisfactory. These findings highlight the limited standalone diagnostic performance of bile cytology in routine clinical practice and suggest that its diagnostic yield may be influenced by both the biological characteristics of cholangiocarcinoma and procedural factors related to specimen acquisition and processing.

The overall sensitivity observed in our study was lower than that reported in previous studies, which have generally described sensitivities ranging from approximately 20% to 60% for bile cytology and biliary brushings. Several biological and methodological factors may account for this finding. First, our cohort included only patients with histologically confirmed cholangiocarcinoma, providing a stringent assessment of cytological performance. In addition, a substantial proportion of patients had perihilar cholangiocarcinoma, which commonly exhibits an infiltrative growth pattern, prominent desmoplastic stroma, and dense fibrosis. These characteristics may limit the exfoliation of malignant cells into the biliary lumen and reduce the cellular yield available for cytological examination. Exfoliated tumor cells may also undergo degeneration during prolonged exposure to bile, further complicating cytological interpretation. Moreover, inflammatory changes related to biliary obstruction, cholangitis, or prior interventions may obscure malignant features and contribute to false-negative or inconclusive results. Finally, variability in specimen collection, preparation, and interpretation inherent in real-world clinical practice may further reduce diagnostic yield.

Collectively, the low sensitivity observed in our cohort likely reflects both the intrinsic biological characteristics of cholangiocarcinoma and procedural variability encountered in routine clinical practice.

An important objective of this study was to compare cytological sensitivity according to the route of specimen acquisition. ERCP is generally used for biliary drainage and tissue acquisition because it provides direct access to the biliary tree and permits brush cytology under fluoroscopic guidance. Nevertheless, ERCP may be technically difficult or unsuccessful in patients with complex hilar strictures, altered gastrointestinal anatomy, or severe biliary obstruction. In such circumstances, PTBD is frequently used for biliary decompression and provides an opportunity to obtain bile for cytological examination. In our cohort, the sensitivity of PTBD-derived cytology was numerically lower than that of ERCP-derived cytology, but the absolute difference was small and did not reach statistical significance. However, the PTBD group included only 13 patients, with a single positive cytology result. Therefore, the absence of a statistically significant difference should not be interpreted as evidence of equivalence between the two sampling methods. Rather, these findings indicate that PTBD-derived bile may provide potentially useful cytological material when PTBD is clinically indicated, while further studies with larger sample sizes are required to determine its diagnostic performance relative to ERCP.

An additional methodological consideration is that the two sampling routes were used in different clinical contexts. ERCP was generally the first-line approach, whereas PTBD was selected for patients with technically challenging anatomy, failed ERCP, or severe hilar obstruction. Consequently, the ERCP and PTBD groups should not be regarded as directly interchangeable populations. Differences in tumor location, degree of obstruction, accessibility of the lesion, prior biliary intervention, or other unmeasured clinical factors may have influenced both the choice of drainage route and the probability of obtaining diagnostic cellular material. This potential for confounding by indication is inherent in the retrospective design and provides another reason to interpret the between-group comparison cautiously. The principal value of the PTBD analysis is therefore not to demonstrate equivalence with ERCP, but to characterize the cytological yield obtainable from PTBD-derived bile in patients for whom percutaneous drainage was clinically required. This interpretation is consistent with the observational nature of the study and avoids attributing differences in cytological yield solely to the sampling technique itself.

Another clinically relevant finding was the high frequency of inconclusive and unsatisfactory cytology results. Nearly two-fifths of specimens failed to provide a definitive cytological interpretation, indicating that non-diagnostic sampling represents an important limitation in addition to the low positivity rate. Several factors may contribute to this phenomenon, including limited exfoliation of tumor cells, cellular degeneration following exposure to bile, and contamination by inflammatory cells, blood products, necrotic debris, or biliary sludge. Variability in specimen collection, transport, slide preparation, and cytological interpretation may further affect specimen quality. In clinical practice, an inconclusive or unsatisfactory result may lead to repeated invasive procedures, additional biliary interventions, delays in establishing a definitive

diagnosis, and postponement of appropriate treatment. Therefore, the diagnostic utility of bile cytology should be considered not only in terms of sensitivity but also according to its ability to provide clinically actionable results. These observations also emphasize the potential importance of standardized specimen acquisition, handling, and processing protocols.

From a clinical perspective, a negative bile cytology result should not be interpreted as evidence against malignancy when the clinical suspicion for cholangiocarcinoma remains high. Cytological findings should instead be interpreted together with clinical presentation, laboratory parameters, and cross-sectional imaging. Additional tissue-acquisition techniques, including forceps biopsy, endoscopic ultrasound-guided sampling, cholangioscopy-guided biopsy, fluorescence in situ hybridization, or molecular diagnostic approaches, may be required when conventional cytology is non-diagnostic. Recent advances in endoscopic and molecular technologies may further improve the evaluation of indeterminate biliary strictures, particularly when used in combination with conventional cytology. However, these techniques require specialized equipment, expertise, and additional healthcare resources and are not universally available. In contrast, bile cytology remains inexpensive, technically straightforward, and readily accessible during routine biliary drainage. Thus, particularly in resource-limited settings, bile cytology may continue to have a practical role as part of a multimodal diagnostic strategy, although its limited sensitivity precludes its use as a standalone diagnostic test.

The present study also has several strengths that support its clinical relevance. All included patients had histopathologically confirmed cholangiocarcinoma, providing a consistent reference standard and reducing the risk of including benign strictures as malignant disease. The study reflects routine practice in a tertiary referral setting where both ERCP and PTBD are used according to clinical need, thereby providing pragmatic information on the performance of bile cytology outside a highly standardized research protocol. In addition, the study did not restrict the assessment to a simple positive-versus-negative classification. Retaining inconclusive, unsatisfactory, inflammatory, and infectious categories allowed the analysis to demonstrate that the limitations of bile cytology extend beyond low sensitivity alone and include a substantial burden of non-diagnostic specimens. Finally, the direct description of PTBD-derived cytology addresses a clinically relevant setting in which evidence remains less extensive than for ERCP-based sampling and may be particularly informative for centers where percutaneous biliary drainage is frequently required.

Several limitations of this study should be acknowledged. First, the retrospective design may have introduced selection bias and limited control over procedural and pathological variables. Second, this was a single-center study, which may limit the generalizability of the findings to other institutions. Third, the relatively small number of patients undergoing PTBD substantially reduced the statistical power to detect modest differences between the two sampling techniques and prevented firm conclusions regarding equivalence. Fourth, variations in specimen acquisition, processing, and cytological interpretation over the study period may have influenced diagnostic performance. Finally, because the study population consisted exclusively of patients with

histologically confirmed cholangiocarcinoma, the analysis was limited primarily to sensitivity and cytological positivity. A further consideration is that requiring histopathological confirmation may have affected the spectrum of patients represented in the cohort. The reported sensitivity therefore applies specifically to patients with ultimately confirmed cholangiocarcinoma who also underwent biliary cytologic sampling and should not be extrapolated directly to all patients presenting with indeterminate biliary strictures. Patients in whom definitive tissue confirmation was not obtained, or in whom biliary sampling was not performed, were outside the analytic population. Although this design strengthens certainty regarding the presence of malignancy, it may also introduce selection related to diagnostic work-up and referral patterns. This feature should be considered when comparing the present findings with studies using broader clinical populations or different reference standards. Specificity, positive predictive value, and negative predictive value could not be reliably assessed in the absence of patients without cholangiocarcinoma.

Future studies should focus on improving the diagnostic yield of biliary cytology through standardized sampling techniques, optimized specimen processing, and integration with adjunctive diagnostic methods. Prospective multicenter studies with larger numbers of patients undergoing PTBD are particularly needed to more precisely define the diagnostic performance of PTBD-derived bile cytology and to determine whether the route of sample acquisition meaningfully influences diagnostic sensitivity. Emerging molecular and imaging technologies may further enhance diagnostic accuracy, but their role should be evaluated within practical multimodal diagnostic algorithms. Overall, our findings reinforce the limited sensitivity of conventional bile cytology and support cautious interpretation of negative or non-diagnostic results, regardless of the biliary drainage approach used.

Conclusions

Bile cytology demonstrated low sensitivity for the diagnosis of cholangiocarcinoma in this real-world cohort. Although ERCP-derived specimens showed numerically higher sensitivity than PTBD-derived specimens, no statistically significant difference was observed. Given the small number of patients undergoing PTBD and the low overall cytological positivity rate, these findings should not be interpreted as evidence of equivalence between the two sampling methods. Negative bile cytology should therefore be interpreted cautiously, and additional tissue-acquisition techniques should be considered when clinical suspicion for malignancy remains high.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Srinakharinwirot University (protocol code SWUEC-147/2564E, approved on 11 October 2021). Informed consent was obtained from all subjects or legal representatives involved in this study.

Acknowledgments

I would like to thank the staff of the Department of Medicine and Radiology, Srinakharinwirot University for their assistance with the data collection.

References

1. Chaudhary U, Shah SL. Advances in endoscopic diagnosis and management of cholangiocarcinoma. *J Clin Med* 2025;14(17):6028. doi:10.3390/jcm14176028
2. Ilyas SI, Khan SA, Hallemeier CL, et al. Cholangiocarcinoma—evolving concepts and therapeutic strategies. *Nat Rev Clin Oncol* 2018;15(2):95-111. doi:10.1038/nrclinonc.2017.157.
3. Valle JW, Kelley RK, Nervi B, et al. Biliary tract cancer. *Lancet* 2021;397(10272):428-44. doi:10.1016/S0140-6736(21)00153-7.
4. European Association for the Study of the Liver. EASL-ILCA clinical practice guidelines on the management of intrahepatic cholangiocarcinoma. *J Hepatol* 2023;79(1):181-208. doi:10.1016/j.jhep.2023.03.010.
5. Matsumoto K, Takeda Y, Onoyama T, et al. Endoscopic ultrasound-guided fine-needle aspiration biopsy: Recent topics and technical tips. *World J Clin Cases* 2019;7(14):1775-83. doi:10.12998/wjcc.v7.i14.1775.
6. Brindley PJ, Bachini M, Ilyas SI, et al. Cholangiocarcinoma. *Nat Rev Dis Primers*. 2021;7(1):65. doi:10.1038/s41572-021-00300-2.
7. Sato T, Nakai Y, Fujishiro M. Current endoscopic approaches to biliary strictures. *Curr Opin Gastroenterol* 2022;38(5):450-60. doi:10.1097/MOG.0000000000000857.
8. Singh A, Gelrud A, Agarwal B. Biliary strictures: Diagnostic considerations and approach. *Gastroenterol Rep (Oxf)* 2015;3(1):22-31. doi:10.1093/gastro/gou072.
9. Facciorusso A, Crinò SF, Ramai D, et al. Comparative diagnostic performance of different techniques for EUS-guided fine-needle biopsy sampling of solid pancreatic masses: A network meta-analysis. *Gastrointest Endosc* 2023;97(5):839-48.e5. doi:10.1016/j.gie.2023.01.024.
10. Rustagi T, Jamidar PA. Endoscopic treatment of malignant biliary strictures. *Curr Gastroenterol Rep* 2015;17(1):426. doi:10.1007/s11894-014-0426-9.
11. Colombo M, Forcignanò E, Da Rio L, et al. Endoscopic management of benign biliary strictures: Looking for the best stent to place. *World J Clin Cases* 2023;11(31):7521-9. doi:10.12998/wjcc.v11.i31.7521.
12. Dumonceau JM, Tringali A, Blero D, et al. Biliary stenting: Indications, choice of stents and results: European Society of Gastrointestinal Endoscopy (ESGE) clinical guideline. *Endoscopy* 2012;44(3):277-98. doi:10.1055/s-0031-1291633.
13. Yarmohammadi H, Covey AM. Percutaneous biliary interventions and complications in malignant bile duct obstruction. *Chin Clin Oncol* 2016;5(5):68. doi:10.21037/cco.2016.10.07.
14. Hafezi-Nejad N, Liddell RP. Percutaneous Biliary Interventions: Clinical Indications, Comparative Effectiveness, Technical Considerations, Complications, and Outcomes. *Gastrointest Endosc Clin N Am* 2022;32(3):493-505. doi:10.1016/j.giec.2022.02.005.
15. Qumseya BJ, Jamil LH, Elmunzer BJ, et al. ASGE guideline on the role of endoscopy in the management of malignant hilar obstruction. *Gastrointest Endosc* 2021;94(2):222-34.e22. doi:10.1016/j.gie.2020.12.035.
16. Koss LG, Melamed MR. Koss' diagnostic cytology and its histopathologic bases. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
17. Navaneethan U, Njei B, Venkatesh PG, et al. Endoscopic ultrasound in the diagnosis of cholangiocarcinoma as the etiology of biliary strictures: A systematic review and meta-analysis. *Gastroenterol Rep (Oxf)* 2015;3(3):209-15. doi:10.1093/gastro/gou057.
18. Burnett AS, Calvert TJ, Chokshi RJ. Sensitivity of endoscopic retrograde cholangiopancreatography standard cytology: 10-y review of the literature. *J Surg Res*. 2013;184(1):304-311. doi:10.1016/j.jss.2013.06.028.
19. Ferrari Júnior AP, Lichtenstein DR, Slivka A, et al. Brush cytology during ERCP for the diagnosis of biliary and pancreatic malignancies. *Gastrointest Endosc* 1994;40(2 Pt 1):140-5. doi:10.1016/s0016-5107(94)70155-5.
20. Facciorusso A, Crinò SF, Gkolfakis P, et al. Diagnostic work-up of bile duct strictures: European Society of Gastrointestinal Endoscopy (ESGE) guideline. *Endoscopy* 2025;57(2):166-85. doi:10.1055/a-2481-7048.
21. Layfield LJ, Ehya H, Filie AC, et al. Utilization of ancillary studies in the cytologic diagnosis of biliary and pancreatic lesions: The Papanicolaou Society of Cytopathology guidelines for pancreatobiliary cytology. *Diagn Cytopathol*. 2014;42(4):351-62. doi:10.1002/dc.23093.
22. De Bellis M, Sherman S, Fogel EL, et al. Tissue sampling at ERCP in suspected malignant biliary strictures (part 1). *Gastrointest Endosc* 2002;56(4):552-61. doi:10.1067/mge.2002.128132.
23. Nayar R, Wilbur DC, eds. The Bethesda System for Reporting Cervical Cytology. 3rd ed. Cham: Springer; 2015.
24. Pitman MB, Layfield LJ. Guidelines for pancreaticobiliary cytology from the Papanicolaou Society of Cytopathology: A review. *Cancer Cytopathol* 2014;122(6):399-411. doi:10.1002/cncy.21427.
25. Bewick V, Cheek L, Ball J. Statistics review 8: Qualitative data—tests of association. *Crit Care* 2004;8(1):46-53. doi:10.1186/cc2428.
26. Cardinale V. Classifications and misclassification in cholangiocarcinoma. *Liver Int* 2019;39(2):260-2. doi:10.1111/liv.13998.
27. Chadwick BE. Beyond cytomorphology: Expanding the diagnostic potential for biliary cytology. *Diagn Cytopathol* 2012;40(6):536-41. doi:10.1002/dc.22839.
28. Ito H, Satoh K, Hamada S, et al. The evaluation of MSX2 mRNA expression level in biliary brush cytological specimens. *Anticancer Res* 2011;31(3):1011-7.

ORIGINAL ARTICLE

Predictive Value of AIMS65 Score for Clinical Outcomes in Thai Cirrhotic Patients with Upper Gastrointestinal Bleeding

Natthawat Aranyawat

Department of Internal Medicine, Nopparat Rajathanee Hospital

KEYWORDS

AIMS65 score; cirrhosis; upper gastrointestinal bleeding; mortality; prediction

CORRESPONDING AUTHOR

Natthawat Aranyawat

Department of Internal Medicine, Nopparat Rajathanee Hospital
679 Ramindra Road, Khanna Yao Sub-district, Khanna Yao District, Bangkok 10230, Thailand
E-mail: natthawat2542@hotmail.com

ABSTRACT

While the AIMS65 score has been proven effective for the general upper gastrointestinal bleeding (UGIB) population, its performance remains uncertain in patients with underlying cirrhosis. Therefore, this study aimed to evaluate the predictive accuracy of the standard AIMS65 cutoff score of ≥ 2 among Thai cirrhotic patients with UGIB across two levels of clinical endpoints: (1) the primary outcome of in-hospital mortality, and (2) secondary outcomes including rebleeding, blood transfusion, and medical resource utilization (length of stay and costs), while also identifying independent clinical predictors associated with each outcome. This retrospective study reviewed 274 adult cirrhotic patients admitted with overt UGIB. Clinical outcomes—including mortality, rebleeding, blood transfusions, length of stay (LOS), and costs—were compared between AIMS65 scores < 2 and ≥ 2 using ROC curves, multivariable logistic regression, and quantile regression. The AIMS65 score demonstrated acceptable predictive ability for mortality in the overall cohort (AUROC 0.75) and the variceal UGIB (VUGIB) subgroup (AUROC 0.76), though performance was limited in non-variceal UGIB (NVUGIB) cases (AUROC 0.69). Multivariable analysis confirmed that the dichotomized AIMS65 score was a significant independent predictor solely for in-hospital mortality (adjusted OR 2.56, 95% CI 1.04–6.31, $p=0.040$). Additionally, quantile regression confirmed that the high-risk group had significantly longer median LOS and higher hospitalization costs. In conclusion, the standard AIMS65 score (≥ 2) is a valuable and rapid initial screening tool to predict in-hospital mortality, length of stay, and costs in Thai cirrhotic patients with UGIB, particularly in VUGIB, though limited in NVUGIB. However, it does not independently predict rebleeding or blood transfusion. Furthermore, serum creatinine, syncope, and variceal status are also key independent predictors of mortality. Therefore, the AIMS65 score should be used cautiously, particularly when NVUGIB is suspected, and alongside the MELD score, serum creatinine levels, and indicators of hemodynamic instability (such as shock or syncope), to optimize risk stratification in this fragile population.

Received: March 26, 2026

Revised: August 15, 2026

Accepted: August 17, 2026

Introduction

Upper gastrointestinal bleeding (UGIB) is a common and urgent gastrointestinal emergency resulting in significant morbidity, mortality, and healthcare resource utilization¹⁻³. International guidelines recommend early risk stratification to facilitate appropriate management and minimize mortality^{4,5}. Several scoring systems, such as the Glasgow-Blatchford score (GBS), Rockall score (RS), and AIMS65 score, have been developed to predict clinical outcomes⁶⁻⁸. However, while GBS relies on time-consuming laboratory parameters to predict the need for clinical interventions, and RS requires advanced endoscopic findings, this study focused strictly on AIMS65 to establish a simple and efficient screening process in emergency settings. This score offers exceptional bedside simplicity and ease of calculation without relying on endoscopy, making it uniquely optimized for rapid, pre-endoscopic mortality

prediction⁹⁻¹¹. Preliminary validation studies demonstrated the effectiveness of the AIMS65 score in predicting mortality, length of stay (LOS), and hospitalization costs in the general UGIB population, where a score of ≥ 2 traditionally represents a high risk of death¹²⁻¹⁶. However, its applicability remains uncertain in patients with cirrhosis, who often face more severe bleeding complications due to underlying liver disease¹⁷⁻¹⁸. This uncertainty is evidenced by varying thresholds identified in previous cirrhotic cohorts: ≥ 1 by Motola-Kuba et al., ≥ 2 by Huynh, and ≥ 3 by Nagaraja et al.¹⁹⁻²¹. In Thailand, Chang et al. validated the AIMS65 score in the general population using a cutoff of ≥ 2 , showing significant differences between low- and high-risk groups regarding mortality, blood transfusion, LOS, and costs²². Nevertheless, that validation was limited to general patients, leaving its performance in the cirrhosis subgroup yet to be determined.

This study aimed to evaluate the predictive accuracy of the standard AIMS65 cutoff score of ≥ 2 among Thai cirrhotic patients with UGIB across two levels of clinical endpoints: (1) the primary outcome of in-hospital mortality, and (2) secondary outcomes including rebleeding, blood transfusion, and medical resource utilization (length of stay and costs), while also identifying independent clinical predictors associated with each outcome.

Materials and Methods

Study design and data collection

A retrospective study was conducted at Nopparat Rajathanee Hospital from 2018 to 2025 by reviewing the medical records of all adult patients (aged >18 years) with cirrhosis admitted due to overt UGIB. Cirrhosis was confirmed by medical history, physical examination, laboratory tests, and suggestive imaging, while bleeding etiology was identified via endoscopy. Exclusion criteria comprised patients whose final diagnosis was not UGIB after evaluation or those with insufficient data to calculate both AIMS65 and MELD scores. Baseline data collected upon admission for all cirrhotic patients included age, sex, cirrhosis etiologies (alcohol, viruses), comorbidities (diabetes, hypertension, dyslipidemia, ischemic heart disease, cerebrovascular disease, chronic kidney disease, malignancy), and medications (antiplatelets, warfarin, NSAIDs). Laboratory parameters (hemoglobin, platelets, albumin, INR, BUN, creatinine, bilirubin) and specific severity indicators (syncope, altered mental status, hemodynamic instability) were recorded. Bleeding etiologies (variceal or non-variceal) were identified. During the admission period, clinical endpoints—including mortality, rebleeding, blood transfusions, length of stay, and hospitalization costs—were tracked and compared between patients with an AIMS65 score < 2 and ≥ 2 .

Outcome and definitions

The primary outcome was in-hospital mortality, defined as any death occurring during hospitalization. Secondary outcomes included rebleeding, the need for blood transfusion, LOS, and hospitalization costs. The AIMS65 score was calculated by assigning 1 point to each of the following components: albumin level < 3 g/dL, INR > 1.5 , altered mental status, systolic blood pressure ≤ 90

mmHg, and age > 65 years²³. In this study, patients were categorized into low-risk (< 2) and high-risk (≥ 2) groups using a standard AIMS65 cutoff score of ≥ 2 . Altered mental status was defined as a Glasgow Coma Scale score < 14 or documented physician findings of disorientation, lethargy, stupor, or coma^{24,25}. Hemodynamic instability was defined as a calculated mean arterial pressure (MAP) < 65 mmHg. Rebleeding was defined as evidence of UGIB recurring after initial resolution, associated with hemodynamic instability or a hemoglobin decrease of > 2 g/dL.

Statistical analysis

Categorical variables were compared using the chi-square test or Fisher's exact test. Continuous variables were reported as mean $\pm$ SD or median (IQR) and analyzed using the independent t-test or Mann-Whitney U test, depending on data distribution. Multivariable logistic regression analysis was performed to identify independent predictors of clinical and resource utilization outcomes. ROC curves and the area under the curve (AUC) were utilized to evaluate the diagnostic performance of the AIMS65 score. Statistical significance was defined as a p -value < 0.05 . All tests were two-tailed, and analyses were conducted using IBM SPSS software (version 31.1), MedCalc (version 23.4.9), and Microsoft Excel 2024.

Results

Baseline characteristics

A total of 274 cirrhotic patients presenting with UGIB were enrolled, of whom 230 (83.9%) were male, with a median age of 51 years. The predominant etiology of cirrhosis was alcohol ($n = 198$, 72.3%), followed by viruses ($n = 76$, 27.7%). Regarding bleeding etiologies, variceal UGIB (VUGIB) occurred in 161 patients (58.8%), predominantly due to esophageal varices (81.4%) followed by gastric varices (18.6%), while non-variceal UGIB (NVUGIB) was identified in 113 patients (41.2%), primarily caused by peptic ulcer (41.6%) and gastritis (38.1%). The AIMS65 score categorized 123 patients (44.9%) as low-risk (score < 2) and 151 (55.1%) as high-risk (score ≥ 2). Significant differences in baseline characteristics were observed between the risk groups regarding age, ischemic heart disease, nonsteroidal anti-inflammatory drug use, all laboratory parameters, syncope, altered mental status, hemodynamic instability, VUGIB status, and both AIMS65 and MELD scores (Table 1).

Table 1 Baseline characteristics of the participants stratified by AIMS65 risk groups

Characteristics	Total (n = 274)	Low-Risk (n = 123)	High-Risk (n = 151)	p-value
Age (years), median (IQR)	51.0 (45.0–59.0)	50.0 (44.0–56.7)	51.1 (46.0–64.5)	<0.001
Male sex	230 (83.9)	105 (85.4)	125 (82.8)	0.528
Comorbidities				
Diabetes Mellitus	60 (21.9)	22 (17.9)	38 (25.2)	0.131
Hypertension	58 (21.2)	24 (19.5)	34 (22.5)	0.546
Dyslipidemia	2 (0.7)	0 (0.0)	2 (1.3)	0.503
Ischemic heart disease	6 (2.2)	0 (0.0)	6 (4.0)	0.034
Chronic kidney disease	20 (7.3)	9 (7.3)	11 (7.3)	0.991
Cerebrovascular disease	3 (1.1)	1 (0.8)	2 (1.3)	0.686
Malignancy	8 (2.9)	1 (0.8)	7 (4.6)	0.062

Table 1 Continued

Characteristics	Total (n = 274)	Low-Risk (n = 123)	High-Risk (n = 151)	p-value
Medications				
Aspirin and/or clopidogrel	8 (2.9)	3 (2.4)	5 (3.3)	0.670
Warfarin	2 (0.7)	1 (0.8)	1 (0.7)	0.884
Non-steroidal anti-inflammatory drug	19 (7.0)	13 (10.6)	6 (4.0)	0.034
Laboratory parameters				
Hemoglobin (g/dL), median (IQR)	8.3 (6.4–9.9)	9.1 (7.5–10.7)	7.4 (5.7–9.1)	<0.001
Platelet count ($\times 10^3$ /mL), median (IQR)	115.5 (72.0–159.0)	118.0 (76.3–159.8)	112.0 (64.0–157.5)	<0.001
Serum Albumin (g/dL), median (IQR)	2.7 (2.3–3.2)	3.2 (2.8–3.5)	2.5 (2.2–2.8)	<0.001
INR, median (IQR)	1.5 (1.3–1.7)	1.3 (1.2–1.4)	1.6 (1.5–2.0)	<0.001
BUN (mg/dL), median (IQR)	22.5 (14.0–38.0)	22.0 (13.5–31.0)	23.0 (15.0–46.8)	<0.001
Serum creatinine (mg/dL), median (IQR)	0.97 (0.71–1.43)	0.8 (0.7–1.0)	1.1 (0.8–1.7)	<0.001
Bilirubin (mg/dL), median (IQR)	1.7 (0.84–3.54)	1.3 (0.7–2.2)	2.15 (1.1–4.4)	<0.001
Syncope	115 (42.0)	36 (29.3)	79 (52.3)	<0.001
Altered mental status	59 (21.5)	3 (2.4)	56 (37.1)	<0.001
Hemodynamic instability	50 (18.2)	5 (4.1)	45 (29.8)	<0.001
VUGIB status	161 (58.8)	64 (52.0)	97 (64.2)	0.042
AIMS65 score, median (IQR)	2.0 (1.0–2.0)	1.0 (0.0–1.0)	2.0 (2.0–3.0)	<0.001
MELD score, median (IQR)	15.0 (10.0–23.0)	12 (8.0–17.0)	20.0 (12.0–27.0)	<0.001

Data are expressed as number (%) unless otherwise specified. Low-Risk group (AIMS65 score < 2) and High-Risk group (AIMS65 score $\geq$ 2). BMI, body mass index; BUN, blood urea nitrogen; INR, international normalized ratio; IQR, interquartile range; VUGIB, variceal upper gastrointestinal bleeding.

In-hospital mortality

The overall mortality rate was 18.2% (50 patients), with higher AIMS65 scores being associated with increased mortality. Specifically, scores of 0, 1, 2, 3, 4, and 5 corresponded to mortality rates of 0%, 11.0%, 18.1%, 37.7%, 46.2%, and 50.0%, respectively. Among non-survivors, 82.0% had VUGIB compared to 18.0% with NVUGIB ($p < 0.001$). The mortality rate was significantly higher in the high-risk group than in the low-risk group (27.8% vs. 6.5%, $p < 0.001$).

Predictive performance of the AIMS65 Score for In-hospital mortality

For the overall cohort, the AIMS65 score demonstrated an AUROC of 0.75 (95% CI: 0.70–0.80; $p < 0.001$) (Figure 1). Based on the Youden index, the optimal cutoff was identified at ≥ 3 , yielding a sensitivity of 54.0% and a specificity of 81.7%. However, at the lower standard threshold of ≥ 2 , sensitivity increased to 84.0% while specificity decreased to 51.3% (Table 3). Multivariable analysis confirmed that the dichotomized AIMS65 score (cutoff ≥ 2) was a significant independent predictor of mortality, with an adjusted OR of 2.56 (95% CI: 1.04–6.31; $p = 0.040$) (Table 4).

When stratified by bleeding etiology, the AIMS65 score performed reliably in the VUGIB subgroup, demonstrating an AUROC of 0.76 (95% CI: 0.69–0.83; $p < 0.001$) (Figure 1). At a cutoff of ≥ 2 , its sensitivity and specificity were 87.8% and 49.2%, respectively (Table 3). This performance was comparable to the MELD score, which yielded an AUROC of 0.77 (95% CI: 0.70–0.83; $p < 0.001$).

In contrast, the AIMS65 score showed limited predictive power in the NVUGIB subgroup, with an AUROC of 0.69 (95% CI: 0.60–0.78; $p = 0.018$) (Figure 1) and a lower sensitivity of 66.7% (specificity: 53.9%) at the same cutoff. Meanwhile, the MELD score remained more effective in this subgroup, achieving an AUROC of 0.79 (95% CI: 0.70–0.86; $p = 0.001$).

Secondary clinical outcomes

In-hospital rebleeding occurred in 47 patients (17.2%), with the high-risk group exhibiting a significantly higher rebleeding rate than the low-risk group (22.5% vs. 10.6%; $p = 0.009$). Although the AIMS65 score was associated with rebleeding in univariable analysis ($p = 0.010$), it was not an independent predictor after adjusting for confounders in the multivariable model ($p = 0.345$) (Table 4).

Regarding blood product usage, a significant majority of patients (82.5%) required blood transfusions. The transfusion rate was significantly higher in the high-risk group compared to the low-risk group (89.4% vs. 74.0%, $p = 0.001$). Similar to the rebleeding findings, the dichotomized AIMS65 score was associated with transfusion requirements in univariable analysis ($p = 0.001$) but failed to remain an independent predictor after multivariable adjustment ($p = 0.365$) (Table 4).

Medical resource utilization

As shown in Table 2, patients in the high-risk group had a significantly longer LOS compared to the low-risk group (4.8 vs. 3.9 days; $p < 0.001$). Due to the significant skewness of the LOS distribution, quantile regression at the 50th percentile was utilized. This analysis demonstrated that the dichotomized AIMS65 score was a significant predictor of LOS ($p = 0.017$), with high-risk patients estimated to

stay 0.837 days longer (95% CI: 0.15–1.52) than their low-risk counterparts. Similarly, hospitalization costs were significantly higher for high-risk patients (45,167 vs. 27,386 THB; $p < 0.001$). Quantile regression further confirmed the dichotomized AIMS65 score as a strong predictor of these expenditures ($p < 0.001$), revealing that high-risk patients incurred an estimated median increase of 17,814 THB (95% CI: 11,482–24,146) in hospital expenses.

Table 2 Clinical outcomes and medical resource utilization stratified by AIMS65 risk groups

Variables	Total (n = 274)	Low-Risk (n = 123)	High-Risk (n = 151)	p-value
Mortality	50 (18.2)	8 (6.5)	42 (27.8)	<0.001
Rebleeding	47 (17.2)	13 (10.6)	34 (22.5)	0.009
Blood transfusion	226 (82.5)	91 (74.0)	135 (89.4)	0.001
LOS (days), median (IQR)	4.3 (2.9–6.1)	3.9 (2.9–5.3)	4.8 (3.1–7.1)	<0.001
Cost (THB), median (IQR)	35,772 (22,801–54,767)	27,386 (17,963–39,869)	45,167 (29,678–69,704)	<0.001

Low-Risk group (AIMS65 score < 2) and High-Risk group (AIMS65 score $\geq$ 2). Cost represents the total hospital expenditure during hospitalization, measured in Thai Baht (THB). LOS, length of stay; IQR, interquartile range.

Table 3 AIMS65 score performance for mortality: Overall cohort and subgroup analysis (VUGIB vs. NVUGIB)

AIMS65 Cutoff	Patient Subgroups	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
≥ 1	Overall	100.0 (92.9–100.0)	22.3 (17.0–28.3)	22.3 (21.1–23.6)	100.0
	VUGIB	100.0 (91.4–100.0)	15.8 (9.8–23.6)	28.9 (27.3–30.5)	100.0
	NVUGIB	100.0 (66.4–100.0)	29.8 (21.2–39.6)	11.0 (9.8–12.3)	100.0
≥ 2	Overall	84.0 (70.9–92.8)	51.3 (44.6–58.1)	27.8 (24.3–31.6)	93.5 (88.3–96.5)
	VUGIB	87.8 (73.8–95.9)	49.2 (39.9–58.4)	37.1 (32.4–42.1)	92.2 (83.6–96.5)
	NVUGIB	66.7 (29.9–92.5)	53.9 (43.8–63.7)	11.1 (7.0–17.2)	94.9 (87.9–98.0)
≥ 3	Overall	54.0 (39.3–68.2)	81.7 (76.0–86.5)	39.7 (31.1–9.0)	88.8 (85.4–91.5)
	VUGIB	56.1 (39.7–71.5)	83.3 (75.4–89.5)	53.5 (41.5–65.1)	84.7 (79.6–88.8)
	NVUGIB	44.4 (13.7–78.8)	79.8 (70.8–87.0)	16.0 (7.7–30.3)	94.3 (90.2–96.8)

VUGIB, Variceal Upper Gastrointestinal Bleeding; NVUGIB, Non-variceal Upper Gastrointestinal Bleeding; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

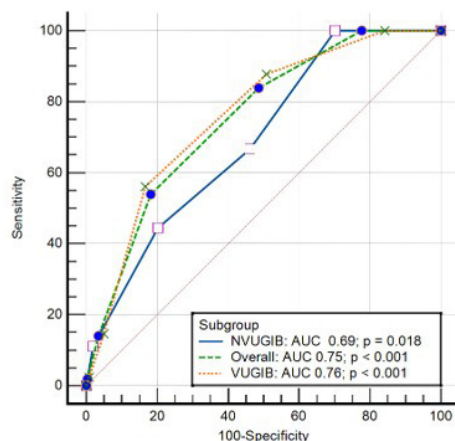


Figure 1 ROC curves of the AIMS65 score for predicting in-hospital mortality across different patient subgroups.

Table 4 Multivariable logistic regression analysis of factors associated with clinical outcomes

Outcomes	Clinical factors	Adjusted OR	95% CI	p-value
Mortality	Serum creatinine	1.91	1.28–2.83	0.001
	Syncope	7.72	3.30–18.09	<0.001
	Hemodynamic instability	0.80	0.33–1.94	0.627
	VUGIB status	4.28	1.79–10.21	0.001
	Dichotomized AIMS65 score	2.71	1.07–6.84	0.035
Rebleeding	Hemoglobin	0.91	0.79–1.04	0.175
	Syncope	1.76	0.89–3.50	0.106
	VUGIB status	1.84	0.91–3.73	0.091
	Dichotomized AIMS65 score	1.16	0.85–1.60	0.345
Blood transfusion	Hemoglobin	0.51	0.42–0.62	<0.001
	Dichotomized AIMS65 score	1.44	0.66–3.15	0.365

CI, confidence interval; Adjusted OR, adjusted odds ratio. Dichotomized AIMS65 score was classified as low-risk (score < 2) and high-risk (score ≥ 2). Variables significantly associated with outcomes in the univariable analysis were included in this model. Age, serum albumin, INR, and altered mental status were excluded to prevent multicollinearity and conceptual overadjustment

Discussion

This study shows that the AIMS65 score is a useful initial screening tool for cirrhotic patients with UGIB in the emergency department, demonstrating acceptable overall mortality prediction (AUROC 0.75). However, its accuracy varies by bleeding etiology. The score performed well in the VUGIB subgroup (AUROC 0.76), comparable to the MELD score (AUROC 0.77) and Huynh's study (AUROC 0.80)²⁰. This efficacy may be because variceal bleeding tends to be sudden and severe, rapidly causing low blood pressure and altered consciousness—two core components of AIMS65. Moreover, since VUGIB usually occurs in advanced cirrhosis, albumin and INR within the score help reflect both acute bleeding severity and baseline liver function.

In contrast, the score showed limited power in NVUGIB cases, with an AUROC of only 0.69. This result differs from general, non-cirrhotic patients reported by Hirai et al. (AUROC 0.83) and Khatua et al. (AUROC 0.94), where AIMS65 is usually much more accurate^{13,14}. In these cases, the MELD score appears more reliable (AUROC 0.79). This low specificity occurs because cirrhosis itself alters the score's components. Chronic liver disease naturally causes low albumin, high INR, and hepatic encephalopathy, independent of acute blood loss. These factors can artificially elevate the baseline score, leading to overestimation of bleeding severity and limited predictive power.

In this study, the MELD score predicted mortality well in both VUGIB and NVUGIB groups. This shows that the survival of cirrhotic patients depends on their background liver function, regardless of where the bleeding comes from. The MELD score uses bilirubin, creatinine, and INR, which reflect liver and kidney failure. Since acute bleeding can quickly worsen these organs, using the MELD score at initial emergency presentation helps doctors assess patient risk more accurately.

Regarding the primary outcome, the VUGIB group had a very high mortality rate, accounting for 82.0% of all deaths. This severity is linked to portal hypertension and advanced cirrhosis. A variceal rupture causes massive, high-pressure

bleeding and rapid blood loss. In a weak cirrhotic liver, the resulting low blood pressure cuts blood flow to the liver, worsening liver failure. Furthermore, poor blood clotting common in cirrhosis (such as low platelets and high INR) makes hemostasis difficult, increasing early death risks. This explains why cirrhotic patients have a nearly three-fold higher mortality rate than general UGIB patients with identical AIMS65 scores, as demonstrated in the Thai cohort by Chang et al²².

Based on the Youden index, the best cutoff to predict death was ≥ 3, which matches the threshold by Nagaraja et al²¹. However, in an emergency department, a "rule-out" strategy is essential to avoid missing high-risk patients. A cutoff of ≥ 3 gives a low sensitivity of 54.0%, which is unsafe. Therefore, this study suggests that using the standard threshold of ≥ 2 is more rational for front-line triage. While the specificity drops to 51.3%, the high sensitivity of 84.0% ensures safety in finding patients at risk of death, accepting the trade-off of some over-triage and higher costs.

Beyond predicting mortality, this study also evaluated secondary outcomes, including rebleeding and blood transfusion. In univariable analysis, high-risk patients (AIMS65 score ≥ 2) had a significantly higher rebleeding rate (22.5% vs. 10.6%) and blood transfusion rate (89.4% vs. 74.0%) than low-risk patients. This indicates that cirrhotic patients are highly fragile and face severe risks of early complications. Notably, low-risk patients in this study had a higher rebleeding rate (10.6%) than the high-risk general UGIB patients (3.7%) reported by Chang et al²². This suggests that cirrhosis increases baseline risks regardless of the initial score. However, in multivariable analysis, the AIMS65 score was not a significant independent predictor for rebleeding and blood transfusion. This implies that acute clinical factors—such as baseline hemoglobin—have a more direct and powerful effect on these outcomes than the AIMS65 score itself.

Furthermore, this study showed that the standard AIMS65 cutoff score (≥2) is a strong predictor of longer length of stay (LOS) and hospital costs. Notably, the median

cost for high-risk cirrhotic patients in this study (45,167 THB) was more than double that of the general UGIB population reported by Chang et al²². This huge cost reflects the clinical reality that treating UGIB in cirrhotic patients requires intense, complex, and expensive care—such as urgent endoscopy, special medications, and targeted blood transfusion.

Based on these clinical realities, this study offers practical insights for front-line triage. While the standard AIMS65 score (≥ 2) remains a rapid, reliable tool for VUGIB, clinicians must exercise strict caution when NVUGIB is suspected due to its limited predictive power in this subgroup. To ensure patient safety and avoid under-triage, AIMS65 screening should not be used as a standalone tool. Instead, it should be combined with the MELD score, serum creatinine levels, and indicators of hemodynamic instability (such as shock or syncope).

This study has several strengths. To the best of our knowledge, this is the first study in Thailand to verify the standard AIMS65 cutoff of ≥ 2 specifically in a cohort of cirrhotic patients with UGIB, evaluating both its prognostic value and clinical application. Furthermore, it has a good study design, high-quality data collection resulting in a reliable dataset, and utilizes appropriate advanced statistical analyses, such as quantile regression, to accurately model skewed LOS and cost data.

However, certain limitations must be acknowledged. First, the single-center nature may limit the generalizability of findings to other clinical settings, meaning they may not be fully representative of the entire Thai population. Second, its retrospective design relied solely on existing medical records, resulting in missing information to explain differences in outcomes and making it impossible to track mortality after discharge. Third, as the LOS in this study was less than 7 days, the hospitalization period may have been too short to adequately assess the difference in re-bleeding rates between the two groups. Fourth, only data on in-hospital mortality were included; deaths after discharge were not recorded and may have led to an underestimation of overall mortality. Lastly, the sample in this study was limited to patients who underwent endoscopy and did not include those managed as outpatients or who declined the procedure. Therefore, these results may not be generalizable to the entire UGIB patient population.

Based on the findings, future research should focus on three main areas. First, large-scale, prospective multi-center studies are recommended to confirm these thresholds and improve the generalizability of the results across different hospitals. Second, future studies could follow patients after hospital discharge to track long-term mortality and late rebleeding events. Lastly, it would be useful to evaluate a modified scoring system by combining AIMS65 with established liver-specific models like the MELD score, or individual clinical predictors such as bilirubin and creatinine, to determine whether this integration can enhance predictive accuracy for fragile cirrhotic patients in emergency settings.

Conclusions

The standard AIMS65 score (≥ 2) is a valuable and rapid initial screening tool to predict in-hospital mortality, length of stay, and costs in Thai cirrhotic patients with UGIB, particularly in VUGIB, though limited in NVUGIB. However, it does not independently predict rebleeding or

blood transfusion. Furthermore, serum creatinine, syncope, and variceal status are also key independent predictors of mortality. Therefore, the AIMS65 score should be used cautiously, particularly when NVUGIB is suspected, and alongside the MELD score, serum creatinine levels, and indicators of hemodynamic instability (such as shock or syncope), to optimize risk stratification in this fragile population.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Nopparat Rajathanee Hospital (protocol number 19/2569; approval date: November 13, 2025). Informed consent was obtained from all participants or their legal representatives.

Acknowledgments

The researcher would like to thank the Research and Technology Evaluation Department of Nopparat Rajathanee Hospital for their kind support and approval to conduct this research study.

References

1. Alali AA, Boustany A, Martel M, et al. Strengths and limitations of risk stratification tools for patients with upper gastrointestinal bleeding: A narrative review. *Expert Rev Gastroenterol Hepatol* 2023;17(8):795-803. doi:10.1080/17474124.2023.2242252.
2. Nigam GB, Oakland K, Hearnshaw S, et al. Acute upper gastrointestinal bleeding in the UK: 2022 audit update. *Gut* 2026;75(4):760-71. doi:10.1136/gutjnl-2025-335134.
3. Orpen-Palmer T, Prentice R, Blatchford O. A review of risk scores within upper gastrointestinal bleeding. *J Clin Med* 2023;12(11):3678. doi:10.3390/jcm12113678.
4. Allo G, Gillesen J, Gülcicegi D, et al. Comparison of lactate clearance with established risk assessment tools in predicting outcomes in acute upper gastrointestinal bleeding. *J Clin Med* 2023;12(7):2716. doi:10.3390/jcm12072716.
5. Gralnek IM, Stanley AJ, Morris AJ, et al. Endoscopic diagnosis and management of nonvariceal upper gastrointestinal hemorrhage: European Society of Gastrointestinal Endoscopy (ESGE) Guideline update 2021. *Endoscopy* 2021;53(3):300-32. doi:10.1055/a-1369-5274.
6. Nasr Isfahani M, Fallah M, Adibi Sedeh P, et al. Predicting mortality and readmission in UGIB: A comparative study of Rockall, Glasgow-Blatchford, and AIMS65 scores. *BMC Gastroenterol* 2025;25(1):727. doi:10.1186/s12876-025-04344-6.
7. Mohammadyari F, Aflak A, ZareDini M, et al. Risk stratification and scoring systems in upper and lower gastrointestinal bleeding: Review of performance and limitations in the emergency department. *Frontiers in Medicine* 2025;12:1564015. doi:10.3389/fmed.2025.1564015.
8. Boğa E. Comparison of rockall and glasgow-blatchford scores in GI bleeding. *Front Healthc Res* 2025;2(2):1-9. doi:10.63918/fhr.v2.n2.p1-9.2025.
9. Kılıç M, Ak R, Dalkılınç Hökenek U, et al. Use of the AIMS65 and pre-endoscopy Rockall scores in the prediction of mortality in patients with the upper gastrointestinal bleeding. *Ulus Travma Acil Cerrahi Derg* 2022;29(1):100-4. doi:10.14744/tjtes.2022.38890.

10. Ak R, Hökenek NM. Comparison of AIMS65 and Glasgow Blatchford scores in predicting mortality in patients with upper gastrointestinal bleeding. *Rev Assoc Med Bras* (1992) 2021;67(5):766-70. doi:10.1590/1806-9282.20210580.
11. Yavuz A, Karabulut Ü, Ebik B, et al. Prognostic value of the neutrophil percentage-to-albumin ratio in acute non-variceal upper gastrointestinal bleeding. *J Clin Med* 2026;15(8):2854. doi:10.3390/jcm15082854.
12. Hirai K, Kikuchi T, Okubo H, et al. AIMS65 predicts prognosis of patients with duodenal ulcer bleeding; a comparison with other risk-scoring systems. *Eur J Gastroenterol Hepatol* 2021;33(4):559-65. doi:10.1097/MEG.0000000000002010.
13. Khatua CR, Mishra E, Behera PS, et al. The AIMS65 score is a superior predictor of in-hospital outcomes in patients with nonvariceal upper gastrointestinal bleeding of benign etiology. *Euroasian J Hepato Gastroenterol* 2026;16(1):45-50. doi:10.5005/jp-journals-10018-1508.
14. Güner NG, Çatal F, Yürümez Y, et al. Comparison of the new risk score (ABL) with the Glasgow Blatchford Score, AIMS65, and pre-endoscopic Rockall Score in patients with upper gastrointestinal bleeding admitted to the emergency department. *BMC Emerg Med* 2025;25(1):131. doi:10.1186/s12873-025-01291-z.
15. Morarasu BC, Morarasu S, Rusu D, et al. Age, blood tests and comorbidities and AIMS65 risk scores outperform Glasgow-Blatchford and pre-endoscopic Rockall score in patients with upper gastrointestinal bleeding. *World J Clin Cases* 2023;11(19):4513-26. doi:10.12998/wjcc.v11.i19.4513.
16. Krishnan D, Ganesh P, Subramanyam S, et al. Comparison of risk scores for predicting in-hospital mortality among patients presenting with acute upper gastrointestinal bleed: A retrospective study from a tertiary care centre, Chennai, India. *J Clin Diagn Res* 2022;16(7):OC04-8. doi:10.7860/JCDR/2022/50158.16585.
17. Franco MC, Jang S, Martins BDC, et al. Risk stratification in cancer patients with acute upper gastrointestinal bleeding: Comparison of glasgow-blatchford, Rockall and AIMS65, and development of a new scoring system. *Clin Endosc* 2022;55(2):240-7. doi:10.5946/ce.2021.115.
18. Lau JYW. The value of risk scores to predict clinical outcomes in patients with variceal and non-variceal upper gastrointestinal bleeding. *Clin Endosc* 2021;54(3):145-7. doi:10.5946/CE.2021.077.
19. Motola-Kuba M, Escobedo-Arzate A, Tellez-Avila F, et al. Validation of prognostic scores for clinical outcomes in cirrhotic patients with acute variceal bleeding. *Ann Hepatol* 2016;15(6):895-901. doi:10.5604/16652681.1222107.
20. Tantai XX, Liu N, Yang LB, et al. Prognostic value of risk scoring systems for cirrhotic patients with variceal bleeding. *World J Gastroenterol* 2019;25(45):6668-80. doi:10.3748/wjg.v25.i45.6668.
21. Nagaraja BS, Shetty S, Shetty A, et al. A comparative study of AIMS 65 score with other scoring systems to predict outcomes in patients with upper GI bleed in chronic liver disease. *Int J Contemp Med Res* 2019;6(5):E5-8. doi:10.21276/ijcmr.2019.6.5.13.
22. Chang A, Ouejiaaphant C, Akarapatima K, et al. Prospective comparison of the AIMS65 score, glasgow-blatchford score, and rockall score for predicting clinical outcomes in patients with variceal and nonvariceal upper gastrointestinal bleeding. *Clin Endosc* 2021;54(2):211-21. doi:10.5946/ce.2020.068.
23. Nik Pa NH, Abu Bakar A, Sabardin DM, et al. Diagnostic performance of AIMS65, glasgow-blatchford, and pre-endoscopy rockall scores in predicting clinical outcomes among upper gastrointestinal bleeding patients in the emergency department. *Cureus* 2025;17(11):e96795. doi:10.7759/cureus.96795.
24. Manji HK, Sawe HR, Kilindimo S, et al. Clinical profile and mortality among adult patients presenting with altered mental status to the emergency departments of a tertiary hospital in Tanzania: A descriptive cohort study. *Pan Afr Med J* 2022;41:46. doi:10.11604/pamj.2022.41.46.31601.
25. Lee HA, Jung HK, Kim TO, et al. Clinical outcomes of acute upper gastrointestinal bleeding according to the risk indicated by Glasgow-Blatchford risk score-computed tomography score in the emergency room. *Korean J Intern Med* 2022;37(6):1176-85. doi:10.3904/kjim.2022.099.

ORIGINAL ARTICLE

Impact of the COVID-19 Pandemic on STEMI Care Processes and Treatment Outcomes in the Emergency Department of a University Hospital

Worapot Khampeera¹, Parinya Tianwibool²

¹ Emergency Unit, Outpatient Department and Emergency Nursing Section, Nursing Department, Maharaj Nakorn Chiang Mai Hospital

² Department of Emergency Medicine, Faculty of Medicine, Chiang Mai University

KEYWORDS

COVID-19; emergency department; impact; patient care process; STEMI

CORRESPONDING AUTHOR

Worapot Khampeera

Emergency Unit, Outpatient Department and Emergency Nursing Section, Nursing Department, Maharaj Nakorn Chiang Mai Hospital

110 Intrawarorot Road, Si Phum Sub-district, Mueang Chiang Mai District, Chiang Mai 50200, Thailand

E-mail: worapot.k@cmu.ac.th

Received: March 11, 2026

Revised: August 15, 2026

Accepted: August 20, 2026

ABSTRACT

This study aimed to assess the impact of the COVID-19 pandemic on emergency department (ED) care processes and clinical outcomes among patients with STEMI at a university hospital in Northern Thailand. This retrospective study reviewed electronic medical records of consecutive adult patients with newly diagnosed STEMI presenting to the ED between April and September of 2019-2021. Study periods were classified as pre-pandemic (2019) and pandemic years (2020-2021). Data collected included patient demographics, mode of arrival, key time-based process indicators (door-to-electrocardiogram [ECG], door-to-diagnosis/fast-track activation, door-to-exit, and diagnosis-to-wire crossing), length of hospital stay, and clinical outcomes. A total of 112 patients were included. The proportion of patients arriving via Emergency Medical Services (EMS) increased significantly during the pandemic (10.81% in 2019; 29.73% in 2020; 34.21% in 2021). Median door-to-ECG consistently met the institutional target of ≤ 5 minutes across all study periods. Door-to-diagnosis/activation and door-to-exit times were longer during the pandemic, particularly in 2021, coinciding with enhanced infection-control measures and COVID-19 screening. Despite the pandemic, the patient care team continued to implement the coronary intervention to ensure patients received prompt cardiac catheterization, and it was found that the direct transfer to the catheterization laboratory increased significantly during the pandemic (0.00% in 2019 vs. 31.60% in 2021). Median length of hospital stays increased from two days in the pre-pandemic period to three days during the pandemic, while clinical improvement was observed in more than 86.50% of patients. In conclusion, during the COVID-19 pandemic, patients with STEMI were more likely to arrive by EMS and experienced longer hospital stays; however, key time-based ED performance indicators, especially door-to-ECG time, were maintained at accepted standards. These findings highlight the importance of preparedness planning, streamlined workflows, and multidisciplinary coordination to preserve life-saving processes during future infectious disease outbreaks.

Introduction

The coronavirus disease 2019 (COVID-19) pandemic profoundly affected healthcare systems and patient care processes across all disease groups, particularly among patients with ST-segment elevation myocardial infarction (STEMI), a condition associated with a high short-term mortality rate if timely treatment is not provided¹. Global studies conducted during the pandemic demonstrated that although the number of patients with STEMI presenting to hospitals decreased by approximately 20% (incidence rate ratio [IRR] 0.80; 95% CI 0.76-0.84; $p < 0.001$)², Those who did present were more likely to have severe clinical conditions, including heart failure, cardiogenic shock, and out-of-hospital cardiac arrest (OHCA)³⁻⁵. Consequently, the

COVID-19 pandemic directly affected the care of this high-risk patient population.

The COVID-19 pandemic posed multiple barriers to ED care processes⁶ for patients with STEMI. A global meta-analysis including more than 87,000 patients from 32 studies found that the time to coronary intervention increased by an average of 8.10 minutes (95% CI 3.90-12.30; $p < 0.001$), and in-hospital mortality increased significantly by 1.27-fold (95% CI 1.09-1.49; $p < 0.05$)³. Notably, low- and middle-income countries experienced an even higher increase in mortality, up to 1.52-fold (95% CI 1.13-2.05; $p < 0.05$)³. These delays resulted from multiple factors, beginning at the access stage, where more extensive history taking was required at triage, such as travel history to high-risk areas or close contact with

individuals infected with COVID-19. Patients with risk factors or unclear and complex histories were classified as patients under investigation (PUI) and required isolation from the general patient population, leading to delays in entry into clinical assessment. In addition, ED healthcare personnel required additional time to wear personal protective equipment (PPE) before patient evaluation, further delaying entry into the care process. These delays were particularly critical for ECG, which is essential for diagnosis and activation of fast-track treatment pathways⁷ within a limited time frame. A study conducted in Thailand⁸ revealed that the duration for diagnosis escalated from 7 to 59 minutes ($p<0.001$), while the interval for coronary intervention extended from 23 to 39 minutes ($p<0.001$) when comparing the pre-pandemic and pandemic periods. A study conducted in Singapore similarly reported that the time to coronary intervention increased from 60 to 69 minutes ($p<0.001$), with most of the delays occurring in the ED⁹. These delays not only subsequently affected patient diagnosis but also affected patient disposition from the ED to undergo primary percutaneous coronary intervention (primary PCI), which represents the ultimate therapeutic goal in managing of patients with STEMI. As a result, treatment timelines failed to meet established standards^{7,9,10}, prolonging myocardial ischemic time and increasing the risk of severe complications, including cardiac arrest and death.

Although the COVID-19 situation has subsided, learning from and reflecting on past experiences remains critically important for preparedness against potential future infectious disease outbreaks. Therefore, this study aimed to investigate the impact of the COVID-19 pandemic on the care processes of patients with STEMI in the ED. This data will be utilized to develop effective clinical practice guidelines for crisis situations, achieve standard care goals, and enhance patient survival.

Materials and Methods

This retrospective study included all consecutive eligible newly diagnosed patients with the ECG showing STEMI who presented to the ED of Maharaj Nakorn Chiang Mai Hospital, a university-level hospital and cardiac center. Two study periods were defined, the pandemic period in Thailand each year^{11–14}: (1) the pre-COVID-19 period, from April to September 2019, and (2) the COVID-19 pandemic period, from April to September 2020–2021. The inclusion criteria were: (1) patients newly diagnosed with STEMI, (2) presentation at the ED during the study period between April and September 2019–2021, and (3) availability of complete medical record data. The exclusion criteria were: (1) patients referred from other hospitals, and (2) incomplete medical records. A total of 112 patients were included, comprising 37 patients in 2019, 37 patients in 2020, and 38 patients in 2021, as illustrated in Figure 1.

The research instrument was a case record form developed to collect data from the electronic medical records of patients with STEMI. It consisted of three sections: (1) patient demographic data, including sex, age, mode of arrival, time of presentation, and COVID-19 testing performed in the ED, (2) the ED care process data, including: (2.1) door-to-ECG time, defined as the time from arrival at the ED to perform an ECG, with a target of within 5 minutes, (2.2) door-to-diagnosis/activation time, defined as the time

from arrival at the ED to diagnosis and activation of the fast-track care system, with a target of within 10 minutes, (2.3) diagnosis-to-needle time, defined as the time from diagnosis to administration of thrombolytic therapy, with a target of within 30 minutes, (2.4) door-to-exit time, defined as the time from arrival to transfer out of the ED, with a target of within 60 minutes, (2.5) diagnosis-to-wire crossing time, defined as the time from diagnosis to coronary wire crossing during cardiac catheterization, with a target of within 90 minutes, (2.6) destination after the ED, and (3) outcome data, including length of hospital stay (LOS) and patient status at hospital discharge. The case record form was submitted to three experts, including an ED physician, an ED registered nurse, and a nursing research expert, to evaluate the face validity of the appropriateness and its comprehensiveness in accordance with standards of care for patients with STEMI.

Data collection was conducted in two phases. (1) Preparatory phase: the research team met with hospital administrators of the ED at the study hospital to explain the study objectives, scope, and data collection procedures. Permission was obtained to access the hospital's electronic medical record system in accordance with relevant regulations and institutional policies. In addition, the case record form was prepared, and a secure, private cloud-based data storage system was established, accessible only to the research team via password protection. (2) Data collection phase: the research team independently collected data directly from the hospital's electronic medical record system through the following procedures: (2.1) screening of all newly diagnosed patients with STEMI who presented to the ED between April and September 2019–2021, corresponding to the pre-pandemic period, the first wave of the pandemic, and the subsequent waves, respectively, (2.2) selection of eligible patients based on the predefined inclusion and exclusion criteria, (2.3) extraction and recording of data from the electronic medical records into the case record form, including patient demographic information, ED care process data, and outcome data, (2.4) verification of data accuracy and completeness by rechecking, identification of abnormal or implausible values, and consultation with the responsible physicians or nurses when data were unclear, and (2.5) conversion of personally identifiable information into unique patient identification codes, with personal identifiers stored separately from the research data in a password-protected cloud system, along with data backup procedures implemented to prevent data loss.

Data analysis in this study comprised (1) analysis of the general characteristics of the study population using descriptive statistics, including frequency, percentage, median, and interquartile range (IQR). Comparisons of patient characteristics across study periods were performed using the Chi-square test, Fisher's exact test, and the Kruskal-Wallis test and (2) analysis of factors associated with the goal achievement of care process was conducted using logistic regression to examine the associations between independent variables and the goal achievement of each care process, with comparisons performed between univariable and multivariable logistic regression analyses¹⁵. The results of the analyses were presented in tabular form and narrative descriptions, with findings discussed in comparison with previous studies and established standard care guidelines.

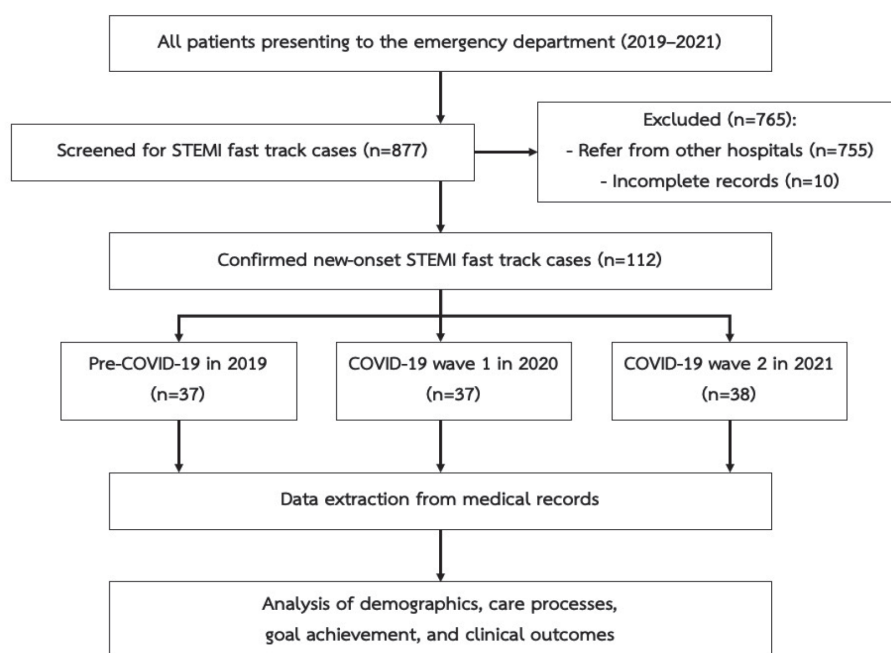


Figure 1 Study flow diagram of STEMI patient selection

Results

Patient Demographics

A total of 112 newly diagnosed patients with STEMI presented to the ED during the study period, including the pre-COVID-19 period in 2019 and the pandemic period in 2020–2021, with an average of 37 patients per year. Detailed characteristics are presented in Table 1. The majority of patients were male (68.42–83.78%), aged over 60 years (59.46–63.16%), and presented to the ED during the day shift (42.11–54.05%). The proportion of patients who arrived at the hospital by walk-in decreased from 89.19% to 70.27% and 65.79% in 2019, 2020, and 2021, respectively. In contrast, patients who arrived by EMS increased significantly from 10.81% to 29.73% and 34.21% in 2019, 2020, and 2021, respectively ($p=0.04$). Most patients transported by EMS were conveyed by non-advanced EMS teams, accounting for 53.57% of all EMS arrivals. Regarding the ED care processes and outcomes data, the median door-to-ECG time ranged from 4.00–5.00 minutes ($p=0.57$). The median door-to-diagnosis/activation time ranged from 8.00–13.00 minutes, with a trend toward longer times during the pandemic period ($p=0.19$). The median diagnosis-to-needle time ranged from 36.00–45.50 minutes ($p=0.59$) among patients receiving thrombolysis ($n=14$). The median door-to-exit time ranged from 66.00–78.00 minutes, with a tendency toward longer times in 2021 ($p=0.44$). The median diagnosis-to-wire crossing time ranged from 119.00 to 124.00 minutes ($p=0.73$). In addition, the proportion of patients directly transferred to the cardiac catheterization laboratory (Cath Lab) increased from 0.00% in 2019 to 5.40% in 2020 and 31.60% in 2021 ($p<0.01$).

The ED Care Process

An analysis of the impact of the COVID-19 pandemic on the care processes of patients with STEMI in the ED

is presented in Tables 2 and 3. The findings indicate that performance against each care target was affected as follows. (1) Door-to-ECG ≤ 5 minutes: In 2020, patients were less likely to achieve the goal compared with 2019 (adjusted OR = 0.37; 95% CI 0.13–1.06; $p=0.06$), with the success rate decreasing from 75.68% to 67.74%. In contrast, in 2021, the likelihood of achieving the goal did not differ from the pre-pandemic period (adjusted OR = 1.08; 95% CI 0.36–3.21; $p=0.89$), with a success rate of 71.05%. (2) Door-to-diagnosis/activation ≤ 10 minutes: In 2020, the likelihood of achieving the goal decreased significantly compared with 2019 (adjusted OR = 0.30; 95% CI 0.10–0.88; $p=0.03$), with the success rate declining from 72.97% to 45.95%. Similarly, in 2021, the likelihood of achieving the target was lower than in 2019 (adjusted OR = 0.46; 95% CI 0.16–1.29; $p=0.14$); however, this difference was not statistically significant, with a success rate of 52.63%. (3) Diagnosis-to-needle ≤ 30 minutes: In both 2020 and 2021, no patients received thrombolytic therapy within 30 minutes, compared with a success rate of 11.11% in 2019. Notably, the number of patients with STEMI receiving thrombolytic therapy in the ED showed a declining trend, decreasing to only 3 and 2 patients in 2020 and 2021, respectively. (4) Door-to-exit ≤ 60 minutes: No statistically significant differences were observed across study years. Although the success rate increased from 43.24% in 2019 to 45.95% in 2020, it subsequently decreased to 42.11% in 2021, due to the hospital's mandatory measures of COVID-19 testing in the ED before hospital admission. (5) diagnosis-to-wire crossing ≤ 90 minutes: No statistically significant differences were observed between study years; however, the success rate demonstrated a declining trend during the pandemic period, decreasing from 37.84% in 2019 to 29.73% in 2020 and 21.05% in 2021, respectively.

An analysis of factors influencing the goal achievement of each step of the care process is presented in the Table 4..

The findings are summarized as follows. (1) Achievement of Door-to-ECG ≤ 5 minutes: Male patients were more likely than female patients to receive an ECG ≤ 5 minutes (adjusted OR = 2.90; 95% CI 1.07-7.86; $p=0.04$). (2) Achievement of Door-to-diagnosis/activation ≤ 10 minutes: Male patients had a higher likelihood of achieving the goal compared with female patients (adjusted OR = 3.47; 95% CI 1.44-10.94; $p=0.01$). In addition, presentation during the evening shift was associated with a higher likelihood of goal achievement compared with the night shift (adjusted OR = 4.97; 95% CI 1.45-17.01; $p=0.01$). (3) Achievement of Door-to-exit ≤ 60 minutes: Presentation during the day shift (adjusted OR = 4.13; 95% CI 1.42-12.01; $p<0.01$) and the evening shift (adjusted OR = 3.98; 95% CI 1.18-13.40; $p=0.03$) was associated with a higher likelihood of achieving the goal compared with the night shift. (4) Achievement of Door-to-

wire crossing ≤ 90 minutes: Patients presenting during the day shift were more likely to successfully undergo primary PCI than those presenting during the night shift (adjusted OR = 4.50; 95% CI 1.41-14.44; $p=0.01$).

Patient Outcomes

According to Table 1, the length of hospital stays increased during the pandemic period, from 2 days in 2019 to 3 days in both 2020 and 2021 ($p=0.02$). The majority of patients (86.50-91.90%) showed clinical improvement with no complication after PCI and were discharged from the hospital ($p=0.81$). During 2021, the subsequent waves, 71.10% of patients with STEMI were tested for COVID-19 before disposition from the ED, and all test results were negative ($p<0.01$).

Table 1 Demographic data of STEMI patients (n=112)

Demographic data	Year 2019 n (%)	Year 2020 n (%)	Year 2021 n (%)	p-value
Sex				
Male	26 (70.27)	31 (83.78)	26 (68.42)	0.26 ^a
Female	11 (29.73)	6 (16.22)	12 (31.58)	
Age (years)				
≤ 60	15 (40.54)	14 (37.84)	14 (36.84)	0.94 ^a
> 60	22 (59.46)	23 (62.16)	24 (63.16)	
Types of arrival				
Walk-in	33 (89.19)	26 (70.27)	25 (65.79)	0.04 ^a
EMS	4 (10.81)	11 (29.73)	13 (34.21)	
Types of EMS (n=28)				
Non-advanced	2 (50.00)	4 (36.36)	9 (69.23)	0.30 ^b
Advanced	2 (50.00)	7 (63.64)	4 (30.77)	
Shift of visit				
Night shift (00:00 - 07:59)	13 (35.14)	8 (21.62)	12 (31.57)	0.72 ^a
Day shift (08:00 - 15:59)	16 (43.24)	20 (54.05)	16 (42.11)	
Evening shift (16:00 - 23:59)	8 (21.62)	9 (24.33)	10 (26.32)	
Door-to-ECG time (min)	4.00	5.00	4.50	0.57 ^c
Median (IQR)	(2.50-6.00)	(2.00-13.00)	(2.00-8.00)	
Door-to-diagnosis/activation time (min)	8.00	13.00	11.50	0.19 ^c
Median (IQR)	(6.00-12.50)	(6.50-33.00)	(5.75-21.00)	
Diagnosis-to-needle time (min) (n=14)	n = 9	n = 3	n = 2	0.59 ^c
Median (IQR)	36.00 (31.00-48.00)	36.00 (32.00-51.00)	45.50 (45.00-46.00)	
Door-to-exit time (min)	67.00	66.00	78.00	0.44 ^c
Median (IQR)	(39.00-91.50)	(46.50-97.50)	(42.75-133.50)	
Diagnosis-to-wire crossing time (min)	119.00	124.00	120.50	0.73 ^c
Median (IQR)	(82.50-190.50)	(89.00-161.00)	(94.00-198.25)	
Disposition				
CCU	37 (100.00)	35 (94.60)	26 (68.40)	$< 0.01^b$
Cath Lab	0 (0.00)	2 (5.40)	12 (31.60)	
Length of stay (day)	2	3	3	0.02 ^c
Median (IQR)	(2.00-3.00)	(2.00-7.50)	(2.00-6.25)	

Table 1 Continued

Demographic data	Year 2019 n (%)	Year 2020 n (%)	Year 2021 n (%)	p-value
Discharge Status				
Improved	34 (91.90)	32 (86.50)	34 (89.50)	0.81 ^b
Not improved	3 (8.10)	5 (13.50)	4 (10.50)	
Swab COVID-19 test (n=27)	n = 0	n = 0	n = 27	
Detected			0 (0.00)	< 0.01 ^a
Not detected			27 (100.00)	

a: Chi-square test; b: Fisher's exact test; c: Kruskal-Wallis test; IQR: Interquartile Range; min: minutes; EMS: Emergency Medical Service; ECG: Electrocardiography

Table 2 The STEMI patient care process's annual goal achievement (n=112)

Care process indicators		Univariable logistic regression analysis			Multivariable logistic regression analysis		
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Door-to-ECG (≤5 min)							
Year	2019	Ref			Ref		
	2020	0.39	0.15-1.03	0.06	0.37	0.13-1.06	0.06*
	2021	0.91	0.33-2.49	0.85	1.08	0.36-3.21	0.89*
Door-to-diagnosis/ activation (≤10 min)							
Year	2019	Ref			Ref		
	2020	0.41	0.16-1.05	0.06	0.30	0.10-0.88	0.03*
	2021	0.49	0.19-1.23	0.13	0.46	0.16-1.29	0.14*
Door-to-exit (≤60 min)							
Year	2019	Ref			Ref		
	2020	1.12	0.45-2.79	0.82	0.98	0.35-2.70	0.96**
	2021	0.96	0.38-2.38	0.92	3.19	0.70-14.50	0.13**
Diagnosis-to-wire crossing (≤90 min)							
Year	2019	Ref			Ref		
	2020	0.70	0.26-1.83	0.46	0.50	0.18-1.43	0.20**
	2021	0.44	0.16-1.22	0.11	0.43	0.07-2.50	0.35**

*: Adjusted by sex, age, types of arrival, shift of visit

** : Adjusted by sex, age, types of arrival, shift of visit, COVID-19 testing

Table 3 Goal achievement following process indicators in the STEMI ED care process

Care process indicators		Goal achievement (%)			p-value
		Year 2019	Year 2020	Year 2021	
1. Door-to-ECG		75.68	67.74	71.05	0.19 ^a
≤ 5 min		(n = 28 / 37)	(n = 21 / 37)	(n = 27 / 38)	
2. Door-to-diagnosis/ activation		72.97	45.95	52.63	0.05 ^a
≤ 10 min		(n = 27 / 37)	(n = 17 / 37)	(n = 20 / 38)	
3. Diagnosis-to-needle		11.11	0.00	0.00	1.00 ^b
≤ 30 min		(n = 1 / 9)	(n = 0 / 3)	(n = 0 / 2)	
4. Door-to-exit		43.24	45.95	42.11	0.94 ^a
≤ 60 min		(n = 16 / 37)	(n = 17 / 37)	(n = 16 / 38)	
5. Diagnosis-to-wire crossing		37.84	29.73	21.05	0.28 ^a
≤ 90 min		(n = 14 / 37)	(n = 11 / 37)	(n = 8 / 38)	

a: Chi-square test; b: Fisher's exact test; ECG: Electrocardiography

Table 4 Factors influencing the goal achievement of the STEMI patient care process

Factors		Univariable logistic regression analysis			Multivariable logistic regression analysis		
		Crude OR	95% CI	<i>p</i> -value	Adjusted OR	95% CI	<i>p</i> -value
Door-to-ECG time (≤ 5 minutes)							
Year	2019	Ref			Ref		
	2020	0.39	0.15-1.03	0.06	0.37	0.13-1.06	0.06
	2021	0.91	0.33-2.49	0.85	1.08	0.36-3.21	0.89
Sex	Female	Ref			Ref		
	Male	1.78	0.75-4.24	0.19	2.90	1.07-7.86	0.04*
Age	≤ 60	Ref			Ref		
	> 60	1.00	0.45-2.23	0.99	1.15	0.49-2.75	0.75
Types of arrival	EMS	Ref			Ref		
	Walk-in	1.93	0.81-4.64	0.14	2.23	0.84-5.91	0.11
Shift of visit	Night	Ref			Ref		
	Day	0.59	0.24-1.49	0.27	0.78	0.29-2.10	0.63
	Evening	1.24	0.40-3.87	0.71	1.96	0.55-6.91	0.30
Door-to-diagnosis / fast track activation time (≤ 10 minutes)							
Year	2019	Ref			Ref		
	2020	0.41	0.16-1.05	0.06	0.30	0.10-0.88	0.03*
	2021	0.49	0.19-1.23	0.13	0.46	0.16-1.29	0.14
Sex	Female	Ref			Ref		
	Male	2.14	0.90-5.08	0.09	3.47	1.44-10.94	0.01*
Age	≤ 60	Ref			Ref		
	> 60	1.64	0.76-3.54	0.21	2.22	0.93-5.26	0.07
Types of arrival	EMS	Ref			Ref		
	Walk-in	1.61	0.68-3.83	0.28	1.74	0.65-4.61	0.27
Shift of visit	Night	Ref			Ref		
	Day	1.11	0.46-2.67	0.81	1.47	0.57-3.84	0.43
	Evening	2.40	0.83-6.88	0.10	4.97	1.45-17.01	0.01*
Door-to-exit time (≤ 60 minutes)							
Year	2019	Ref			Ref		
	2020	1.12	0.45-2.79	0.82	0.98	0.35-2.70	0.96
	2021	0.96	0.38-2.38	0.92	3.19	0.70-14.50	0.13
Sex	Female	Ref			Ref		
	Male	1.38	0.58-3.28	0.46	2.00	0.74-5.38	0.17
Age	≤ 60	Ref			Ref		
	> 60	2.13	0.96-4.72	0.06	2.25	0.94-5.37	0.07
Types of arrival	EMS	Ref			Ref		
	Walk-in	1.92	0.78-4.73	0.16	2.39	0.87-6.55	0.09
Shift of visit	Night	Ref			Ref		
	Day	2.89	1.13-7.37	0.03*	4.13	1.42-12.01	$< 0.01^{**}$
	Evening	2.48	0.85-7.23	0.10	3.98	1.18-13.40	0.03*

Table 4 Continued

Factors		Univariable			Multivariable		
		logistic regression analysis			logistic regression analysis		
		Crude OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Diagnosis-to-wire crossing time (≤ 90 minutes)							
Year	2019	Ref			Ref		
	2020	0.70	0.26-1.83	0.46	0.50	0.18-1.43	0.20
	2021	0.44	0.16-1.22	0.11	0.43	0.07-2.50	0.35
Sex	Female	Ref			Ref		
	Male	1.43	0.54-3.78	0.47	1.68	0.58-4.82	0.34
Age	≤ 60	Ref			Ref		
	> 60	1.13	0.49-2.62	0.78	1.02	0.42-2.50	0.96
Types of arrival	EMS	Ref			Ref		
	Walk-in	0.84	0.34-2.13	0.72	0.75	0.27-2.08	0.58
Shift of visit	Night	Ref			Ref		
	Day	3.79	1.26-11.41	0.02*	4.50	1.41-14.44	0.01*
	Evening	1.96	0.54-7.07	0.30	2.47	0.64-9.46	0.19

*: p -value < 0.05 ; **: p -value < 0.01

Discussion

The care process for patients with STEMI in the ED begins with access to care, triage, and progression through diagnostic and therapeutic pathways, all of which require rapid execution at every step to achieve optimal clinical outcomes^{1,7}. The COVID-19 pandemic had a significant impact on the care processes for patients with STEMI in the ED, affecting both patients' modes of hospital arrival and the duration of care delivery across key stages of care. Nevertheless, the ED service systems were able to adapt and maintain the quality of care across several key performance indicators (KPIs), despite the hospital's mandatory measures for infection control.

In terms of access to care, a significant increase in the proportion of patients with STEMI presenting to the hospital via the EMS system was observed during the COVID-19 pandemic. This finding is consistent with previous studies conducted in Thailand¹⁶ and internationally¹⁷, which reported similar trends. A key contributing factor was the implementation of governmental public health measures, especially the curfew orders and travel restrictions¹⁸, which necessitated greater reliance on EMS for hospital access. In the study setting, multiple basic-level EMS teams were widely distributed, resulting in the majority of patients being transported by basic-level rather than advanced-level EMS teams. Regarding the triage process, during the pandemic, infection risk screening at triage was required, including detailed history taking and additional risk assessment, such as exposure to confirmed cases or travel from high-risk areas. Consequently, some patients not only experienced prolonged screening times but also had to wait in isolation areas, leading to delays in subsequent assessment and treatment processes. This delay was particularly evident among patients classified as PUI, for whom additional time was required for healthcare personnel to wear advanced-level PPE before assessing patients.

When investigating the care processes in the ED, the door-to-ECG time tended to be prolonged during the COVID-19 pandemic, consistent with findings from international studies^{19,20}. The primary reasons are likely attributable to the additional time required for wearing advanced-level PPE and the enhanced infection risk screening procedures compared with normal practice, resulting in delays in initial patient assessment. However, simplification of clinical workflows and increased proficiency in PPE wearing among healthcare personnel during the subsequent waves in 2021 helped mitigate delays in this step. Additionally, male patients were more likely than female patients to achieve an ECG within 5 minutes, which may reflect limitations in the treatment area of the ED and requirements related to patient privacy. Nevertheless, the median ECG accession times across all three study years largely remained within the hospital's KPI (≤ 5 minutes). These findings compare favorably with a study from Taiwan²⁰, which reported a door-to-ECG time average of 6.2 minutes before the pandemic, increasing to 8.5 minutes during the pandemic. The observed performance also exceeded the standards recommended by the Thai ACS 2020⁷ and ESC 2023¹⁰ guidelines (≤ 10 minutes). This demonstrates the effectiveness of the patient care process in maintaining quality standards even during a crisis situation. Contributing factors include intensive staff training to improve PPE wearing proficiency, simulation-based drills, and refinement of infection risk screening processes to enhance operational efficiency.

Regarding door-to-diagnosis/activation time, a trend toward delay was observed during the pandemic, which was associated with initial delays in the door-to-ECG step. Additional contributing factors included workforce limitations and increased physician workload, as clinicians were required to manage multiple patients simultaneously. Furthermore, male patients and those presenting during the

evening shift had a higher likelihood of achieving diagnosis and fast-track activation within 10 minutes. This finding is consistent with a Taiwanese study²⁰ indicating that the shift of arrival influences the efficiency of care processes. These results reflect greater staffing availability and system readiness during the evening shift compared with the night shift. In the study hospital, clearly designated service areas and after-hours clinics were available throughout the evening shift to accommodate non-urgent patients, and staffing levels were also more adequate compared with the night shift, during which only the ED remained open and was supported by fewer healthcare personnel and specialist physicians. This underscores the importance of appropriate human resource management to maintain standards of care for patients with STEMI.

The number of patients with STEMI receiving thrombolytic therapy in the ED markedly decreased. This trend aligns with international practice favoring primary PCI as the gold standard treatment, which yields superior clinical outcomes^{7,10,21}. Therefore, the reduction in thrombolytic therapy should not be interpreted as a system failure, but rather as a reflection of an evolving treatment strategy emphasizing access to advanced reperfusion therapy over pharmacological intervention.

During the COVID-19 pandemic, patients experienced prolonged length of stay in the ED, particularly in 2021, in subsequent waves. The primary contributing factor was COVID-19 testing using the RT-PCR method, which required relatively long waiting times for reporting results before patients could be transferred to the Cath Lab or the cardiac intensive care unit. However, for patients managed under the fast-track system, specimen collection and coordination with the laboratory were initiated early in the care process to expedite test reporting and facilitate the transfer process as rapidly as possible. Patients presenting during the day and evening shifts had a higher likelihood of being transferred out of the ED more rapidly than those presenting during the night shift, which was characterized by more limited resources. This finding is consistent with the report by Khaleghparast et al.²², which indicated that both human resource allocation and support system management across work shifts influence waiting times for patients with STEMI.

Among patients treated with coronary reperfusion via PCI, the study found no significant difference in the median diagnosis-to-wire crossing time between the pre-pandemic and pandemic periods. Nevertheless, the overall median time did not meet the 90-minute target recommended by both national and international guidelines^{7,10}. Delays observed in 2021 may partly be attributed to hospital measures requiring RT-PCR testing for COVID-19 at the ED before hospital admission, a necessary and unavoidable safety measure. Patients receiving care during the day shift were more likely to have successful primary PCI within 90 minutes than those presenting during the night shift, due to greater availability of staff, multidisciplinary teams, and support systems.

Overall outcomes demonstrated that, during the COVID-19 pandemic, a significantly higher proportion of patients with STEMI were transferred directly from the ED to the Cath Lab. Although hospital length of stay increased due to waiting for COVID-19 test results, most patients demonstrated clinical improvement, and no COVID-19 infection was detected. These outcomes reflect improvements in care planning by multidisciplinary teams and sustained

interprofessional collaboration. Ongoing simulation training for STEMI fast-track care enabled the hospital to maintain performance in KPI despite the implementation of stringent infection control measures.

This study has several limitations. As a retrospective study conducted at a single university hospital, the generalizability of the findings to other healthcare settings may be limited. Additionally, the relatively small sample size and the limited number of events for certain indicators, such as thrombolytic administration, may have affected the precision of the statistical analyses. Future studies should consider increasing the sample size by extending data collection during similar emerging infectious disease outbreaks or by conducting multicenter studies to enhance the statistical power and generalizability of the findings, including expanding the scope of the study to determine the impact on patient outcomes. Based on the study findings, the authors propose several recommendations to enhance preparedness for future emerging infectious diseases, including (1) appropriate workforce management and activation systems, particularly during night shifts, (2) development of rapid infection screening systems through streamlined history taking that does not introduce delays, along with investment in rapid diagnostic testing, (3) strengthening staff capacity through simulation-based training, enhanced proficiency in PPE utilization, and support for the nursing role as a central coordinator facilitating collaboration among multidisciplinary teams, and (4) system redesign to ensure that critical standards of timely treatment are maintained while balancing infection safety requirements.

Conclusions

The COVID-19 pandemic significantly affected the care processes for patients with STEMI in the ED, including access to care, infection risk screening, and treatment procedures. During the pandemic, a greater proportion of patients arrived at the hospital via EMS and were referred for coronary intervention. Meanwhile, delays were observed in certain care steps, particularly in diagnosis and activation of the fast-track system. Nevertheless, the healthcare system demonstrated adaptability and maintained the quality of several KPIs despite stringent infection control constraints.

Ethical statement

This study was approved by the Institutional Human Research Ethics Committee of Faculty of Medicine, Chiang Mai University (study code: EME-2565-08881; approval date: March 7, 2022). As this was a retrospective study using existing medical records, informed consent was not obtained.

References

1. Abreu LM. Time is muscle. *Arq Bras Cardiol* 2019;112(4): 408-9. doi:10.5935/abc.20190059.
2. Sofi F, Dinu M, Reboldi G, et al. Worldwide differences of hospitalization for ST-segment elevation myocardial infarction during COVID-19: A systematic review and meta-analysis. *Int J Cardiol* 2022;347:89-96. doi:10.1016/j.ijcard.2021.10.156.
3. Chew NWS, Ow ZGW, Teo VXY, et al. The global effect of the COVID-19 pandemic on STEMI care: A systematic review and meta-analysis. *Can J Cardiol* 2021;37(9):1450-9. doi:10.1016/j.cjca.2021.04.003.

4. De Rosa S, Spaccarotella C, Basso C, et al. Reduction of hospitalizations for myocardial infarction in Italy in the COVID-19 era. *Eur Heart J* 2020;41(22):2083-8. doi:10.1093/eurheartj/ehaa409.
5. Garcia S, Albaghdadi MS, Meraj PM, et al. Reduction in ST-segment elevation cardiac catheterization laboratory activations in the United States during COVID-19 pandemic. *J Am Coll Cardiol* 2020;75(22):2871-2. doi:10.1016/j.jacc.2020.04.011.
6. The Healthcare Accreditation Institute. Hospital and healthcare standards. 5th ed. Nonthaburi: The Healthcare Accreditation Institute (Public Organization); 2022. (in Thai)
7. The Heart Association of Thailand under the Royal Patronage of H.M. the King. Thai acute coronary syndromes guidelines 2020. Samut Prakan: Next Step Design; 2020. (in Thai)
8. Lertsanguansinchai P, Chokesuwattanaskul R, Limjaroen T, et al. Clinical characteristics and in-hospital mortality in patients with STEMI during the COVID-19 outbreak in Thailand. *Biomedicines*. 2022;10(11):2671. doi:10.3390/biomedicines10112671.
9. Chew NW, Sia CH, Wee HL, et al. Impact of the COVID-19 pandemic on door-to-balloon time for primary percutaneous coronary intervention: Results from the Singapore Western STEMI Network. *Circ J* 2021;85(2):139-49. doi:10.1253/circj.CJ-20-0800.
10. Byrne RA, Rossello X, Coughlan JJ, et al. 2023 ESC guidelines for the management of acute coronary syndromes. *Eur Heart J* 2023;44(38):3720-826. doi:10.1093/eurheartj/ehad191.
11. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report-23 [Internet]. Geneva: World Health Organization; 2020 Feb [cited 2026 Aug 15]. Available from: <https://apps.who.int/iris/handle/10665/330992>.
12. World Health Organization. Coronavirus disease 2019 (COVID-19) situation report-200 [Internet]. Geneva: World Health Organization; 2021 Sep [cited 2026 Aug 15]. Available from: https://cdn.who.int/media/docs/default-source/searo/thailand/2021_09_09_eng-sitrep-200-covid19.pdf?sfvrsn=ef31e04e_5.
13. World Health Organization. WHO Thailand weekly situation update no. 222 [Internet]. Geneva: World Health Organization; 2022 Feb [cited 2026 Aug 15]. Available from: https://reliefweb.int/sites/reliefweb.int/files/resources/2022_02_09_tha-sitrep-222-covid-19.pdf.
14. World Health Organization Thailand. WHO Thailand weekly situation update no. 222 [Internet]. Bangkok: World Health Organization; 2022 Feb [cited 2026 Aug 15]. Available from: https://cdn.who.int/media/docs/default-source/searo/thailand/2022_02_09_tha-sitrep-222-covid19_th.pdf?sfvrsn=30e5a084_5.
15. Bursac Z, Gauss CH, Williams DK, et al. Purposeful selection of variables in logistic regression. *Source Code Biol Med* 2008;3:17. doi:10.1186/1751-0473-3-17.
16. Taja S, Tianwibool P, Sutham K, et al. Impact of the COVID-19 pandemic on emergency medical service operation time at Maharaj Nakorn Chiang Mai Hospital. *J Health Syst Res* 2024;18(1):136-47. (in Thai)
17. Abuzeyad FH, Chomayil Y, Amin MI, et al. Effects of COVID-19 on STEMI patients: Single-center experience. *Heart Views* 2022;23(3):138-43. doi:10.4103/heartviews.heartviews_40_22.
18. Hfocus [Internet]. Bangkok: Hfocus News Agency; 2021. The Center for COVID-19 Situation Administration (CCSA) has order a curfew in 10 provinces designated as high-risk areas, effective July 12, 2021. Meanwhile, restrictions on inter-provincial movement will begin in July 10; 2021 [cited 2025 Dec 15]; [about 3 screens]. Available from: <https://www.hfocus.org/content/2021/07/22192>.
19. Bruoha S, Yosefy C, Gallego-Colon E, et al. Impact in total ischemic time and ST-segment elevation myocardial infarction admissions during COVID-19. *Am J Emerg Med* 2021;45:7-10. doi:10.1016/j.ajem.2021.02.020.
20. Hsu CL, Hung SH, Munkhtogoo D, et al. The impact of the COVID-19 pandemic on emergency care of acute myocardial infarction: Findings from the Taiwan Clinical Performance Indicator. *Int J Qual Health Care* 2023;35(2):mzad034. doi:10.1093/intqhc/mzad034.
21. Ibanez B, James S, Agewall S, et al. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: The task force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018;39(2):119-77. doi:10.1093/eurheartj/ehx393.
22. Khaleghparast S, Maleki M, Noohi F, et al. Outcomes of STEMI patients in COVID-19 pandemic: A systematic review and meta-analysis. *Iran J Public Health* 2024;53(9):1964-75. doi:10.18502/ijph.v53i9.16451.

ORIGINAL ARTICLE

Analysis of Patients Coded for Cutaneous Nontuberculous Mycobacterial Infections (ICD-10 A31.1) Using Thai Inpatient Data from 2020 to 2024

Lapasrada Pattarapreeyakul¹, Sunisa Kanchanasutthiyakorn¹, Pichanee Watthanasiri¹, Rujiraporn Pitaksalee¹, Kan Khemnak², Sarrote Boonkerd², Pramet Kaewmesri³, Monpat Chamnanphon⁴, Surakameth Mahasirimongkol⁵

¹ Medical Life Sciences Institute, Department of Medical Sciences, Ministry of Public Health

² Information and Communication Technology Center, Office of the Permanent Secretary, Ministry of Public Health

³ Geo-informatics and Space Technology Development Agency

⁴ Department of Pathology, Faculty of Medicine, Srinakharinwirot University

⁵ Technical and Planning Division, Department of Thai Traditional and Alternative Medicine, Ministry of Public Health

KEYWORDS

Nontuberculous mycobacterial infection (NTM); cutaneous NTM; skin and soft tissue; ICD-10; epidemiological study

CORRESPONDING AUTHOR

Lapasrada Pattarapreeyakul

Department of Medical Sciences, Ministry of Public Health

88/7 Bamrungmuang Road, Tivanon Sub-district, Muang District, Nonthaburi 11000, Thailand

E-mail: lapasrada.m@dmsc.mail.go.th

Received: June 30, 2026

Revised: August 26, 2026

Accepted: August 27, 2026

ABSTRACT

Extrapulmonary nontuberculous mycobacterial (NTM) infections, including cutaneous disease (ICD-10 A31.1), are increasing globally, whereas national epidemiological data remain limited, particularly in Thailand. This retrospective study aimed to characterise the demographic features, geographical distribution, and therapeutic patterns of cutaneous NTM using Thai national inpatient data from electronic medical records in the Health Data Center, Ministry of Public Health, between 2020 and 2024. A total of 103 inpatient records coded as A31.1 were identified. The findings demonstrated a notable upward trend in the number of A31.1 cases across the study period. The cohort had a mean age of 52.1 years and a slight female predominance (52.4%). Among cases with available data, 89.5% had a normal or underweight BMI, and 20.8% were employed in occupations associated with environmental exposure, such as agriculture, forestry, and fisheries. Geographically, the Northeastern region exhibited the highest regional burden, with Khon Kaen accounting for the largest number of provincial-level cases. Therapeutic analysis showed that treatment was predominantly based on multidrug regimens incorporating rifampicin and ethambutol alongside a macrolide. In this cohort, azithromycin was the most frequently prescribed macrolide and was commonly co-prescribed with amikacin or levofloxacin. These findings indicate an emerging public health concern, characterised by a rising incidence and distinct spatial patterning. The study highlights the need for strengthened clinical recognition, improved accuracy and completeness of ICD-10 coding in data entry, and targeted public health strategies to address cutaneous NTM in Thailand.

Introduction

Nontuberculous mycobacteria (NTM) are defined as mycobacterial species other than those belonging to the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*^{1,2}. These organisms are ubiquitous in the environment, commonly found in water sources and soil³. The majority of NTM infections manifest as pulmonary disease. However, extrapulmonary infections can occur in multiple organs, including the skin, soft tissue, and bone, particularly in immunocompromised hosts. Notably, Bacillus Calmette-Guérin (BCG) vaccination confers limited prophylactic protection⁴. Therefore, environmental exposure and immunocompromised status are considered significant risk factors for NTM infection^{5,6}. Moreover, cosmetics and medical procedures carry a risk of contamination in cutaneous NTM infection⁷. Likewise, advanced age and reduced body mass index (BMI) have also been associated

with increased susceptibility to NTM infection in previous studies⁸.

Clinical manifestations of NTM infection demonstrate a broad spectrum, ranging from asymptomatic presentation and non-specific symptoms to severe conditions⁹. NTM are classified as either slow-growing or rapid-growing species based on a seven-day culture growth threshold¹. Clinically significant slow-growing NTM, which typically require more than seven days for colony formation, include *Mycobacterium avium* complex (MAC), *M. kansasii*, *M. marinum*, and *M. ulcerans*. In contrast, rapid-growing species, which produce visible colonies within seven days, include *M. abscessus* complex (MABC), *M. fortuitum* complex, and *M. chelonae*. Focusing on skin and soft tissue NTM infections, *M. abscessus* complex, *M. chelonae*, and *M. fortuitum* complex are the common causes².

The diagnosis of NTM infection requires correlation between clinical manifestations, microbiological findings, and radiographic examinations. According to the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) guidelines, the diagnosis of pulmonary NTM disease necessitates at least two positive sputum cultures obtained at separate time points. In contrast, extrapulmonary NTM infections are diagnosed based on samples from the affected site¹⁰. For instance, suspected lymphadenitis and cutaneous infections require tissue biopsy for histopathological examination and culture, while disseminated NTM disease requires blood cultures for microscopy and mycobacterial culture¹¹. Currently, NTM treatment employs multiple antibiotic regimens, typically combining ethambutol and rifampicin with macrolides such as azithromycin or clarithromycin¹². However, relapse and reinfection may occur following completion of primary treatment^{13,14}.

The incidence of NTM infection has increased globally, as evidenced by systematic reviews and meta-analyses. However, comprehensive global and regional surveillance systems have yet to be fully established^{15,16}. In Thailand, surveillance studies of cutaneous NTM infections are particularly limited. A single-centre study conducted at a university hospital in Bangkok identified 88 cases diagnosed through histopathological examination, microbiological culture, and polymerase chain reaction (PCR) between 2011 and 2017. The majority of cutaneous NTM patients in this cohort presented with one to two skin lesions, most commonly manifesting as nodules, plaques, papules, and abscesses¹⁷.

The microbiological component of the ATS diagnostic criteria, as well as International Classification of Diseases (ICD) codes (ninth and tenth revisions), have been evaluated and demonstrated high predictive value for NTM pulmonary

disease (NTM-PD). While ICD codes provide a useful indicator of true disease burden, they tend to underestimate actual disease prevalence¹⁸. Despite this limitation, ICD-10 codes represent a valuable tool for identifying cases of extrapulmonary NTM infection in large-scale epidemiological studies. Extrapulmonary NTM infections, including cutaneous manifestations, have received considerably less attention in clinical research compared with pulmonary disease, and comprehensive surveillance data remain lacking, particularly in resource-limited settings.

This study employed ICD-10 codes to identify cases of cutaneous nontuberculous mycobacterial (NTM) infection within the Thai national health database. The utilisation of health information systems incorporating ICD-10 coding facilitates comprehensive characterisation of patients categorised under code A31.1, including their demographic and clinical profiles, geographical distribution, and antibiotic utilisation patterns. These data are of considerable value for epidemiological surveillance and for informing evidence-based public health strategies. Moreover, investigations of this nature provide critical insights that support the development of standardised clinical practice guidelines for extrapulmonary NTM diseases in the future.

Materials and Methods

Study design and data source

This retrospective, population-based study utilised electronic medical records as secondary data from the Thai Health Database (43 files) of the Ministry of Public Health (MOPH), collected between January 2020 and December 2024. The database contains individual-level demographic, diagnostic, and clinical information from hospitals nationwide. Data extraction and analysis were conducted at the Department of Medical Sciences, Ministry of Public Health, Thailand (Figure 1).

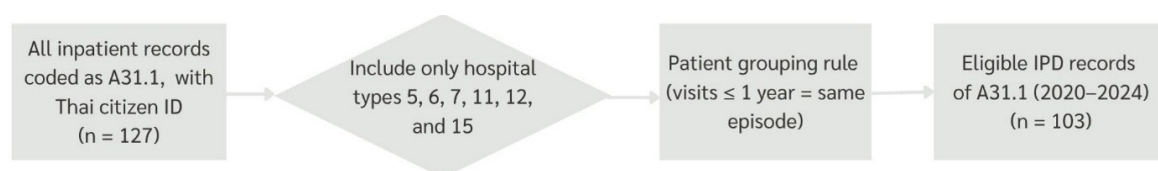


Figure 1 Workflow for Case Identification and Data Processing for Cutaneous NTM. Electronic medical records for all inpatients assigned ICD-10 code A31.1 and holding a valid Thai citizen identification number were extracted across six hospital categories: central hospitals (code 5), general hospitals (code 6), community hospitals (code 7), non-NHSO affiliated hospitals (code 11), non-MOPH affiliated hospitals (code 12), and private hospitals (code 15). Each patient was counted as a single case per calendar year, regardless of multiple visits. A total of 103 unique patient records were included for analysis.

Case definition

Data on cutaneous nontuberculous mycobacterial (NTM) infections were retrieved from the Thai Health Database (43 files) managed by the Ministry of Public Health. Cases were identified using the International Classification of Diseases, Tenth Revision (ICD-10) code A31.1^{19,20}, corresponding to cutaneous NTM infection, recorded between January 2020 and December 2024. To ensure diagnostic reliability, only records from six hospital categories, including central, general, community, non-NHSO-affiliated, non-MOPH-affiliated, and private hospitals, were included. Repeated visits within one year were treated as the same patient episode, while visits separated by more than one year were considered new

episodes. The date of the initial visit in each episode was designated as the baseline date and was not updated unless a new episode was initiated.

Occupation

Occupational information was categorised into nine predefined groups based on the International Standard Classification of Occupations (ISCO). For the purpose of exposure assessment, individuals employed in agriculture, forestry, and fisheries occupations were classified as having environmental exposure, while those in all other occupational categories were classified as having non-environmental exposure (Table 1).

Table 1 Occupational Information Classified into Nine Categories

Classification	Definition	Exposure
1	Managers	Non-environmental exposure
2	Professionals	Non-environmental exposure
3	Technicians and Associate Professionals	Non-environmental exposure
4	Clerical Support Workers	Non-environmental exposure
5	Service and Sales Workers	Non-environmental exposure
6	Skilled Agricultural, Forestry and Fishery Workers	Environmental exposure
7	Craft and Related Trades Workers	Non-environmental exposure
8	Plant and Machine Operators, and Assemblers	Non-environmental exposure
9	Elementary Occupations	Non-environmental exposure

Geographical distribution

Geographical analysis was conducted based on patients' residential addresses, aggregated at the provincial level. For regional comparison, Thailand was divided into six administrative regions: Central, Northern, Northeastern, Eastern, Southern, and Western.

Clinical features

Clinical information was retrieved from the chief complaint section of medical records, which contained physicians' narrative descriptions of presenting symptoms, underlying conditions, estimated incubation periods, follow-up notes, and other relevant clinical observations. Owing to the absence of a standardised recording structure, the extracted data were unstructured and varied across hospitals. Details relating to symptoms and incubation periods were manually reviewed, and free-text descriptions of chief complaint symptoms were subsequently harmonised into predefined clinical categories to enable systematic analysis.

Statistical analysis

Descriptive statistics were applied to summarise demographic characteristics, clinical features, occupational groups, and geographical distribution. Continuous variables were reported as means or medians as appropriate, while categorical variables were presented as frequencies and percentages. Poisson regression was employed to compare annual patient counts, and geographical variation was assessed using a chi-square goodness-of-fit test. A *p*-value less than 0.05 was considered statistically significant.

Results

Cutaneous NTM patient characteristics

A total of 103 cutaneous nontuberculous mycobacterial (NTM) cases were identified from inpatient department records from 53 hospitals between January 2020 and December 2024. The cohort comprised 54 females (52.4%; 95% CI: 42.8%–62.1%) and 49 males (47.6%; 95% CI: 37.9%–57.2%), with a mean age of 52.1 years (median: 57 years). Age distribution showed that the majority of cases occurred in individuals aged 40–60 years (36.89%; 95% CI: 28.2%–46.5%) and 60–80 years (37.86%; 95% CI: 29.1%–47.5%). Younger age groups (0–20 years and 20–40 years) accounted for a smaller proportion of cases. BMI data were available for 67 cases. Of these, the proportion of individuals classified as underweight and those within the normal BMI range were identical at 30 cases (44.8% each)²¹. In contrast, overweight status and obesity were less common, accounting for 6% and 4.5% of cases, respectively (Table 2).

The number of cutaneous NTM IPD cases began at 20 in 2020, decreasing to 13 in 2021. From 2022 onwards, there was a sustained rise, with case counts increasing to 23 in 2022, peaking at 24 in 2023, and remaining stable at 23 cases in 2024. Overall, the cumulative number of A31.1 IPD admissions over the five-year period was 103 cases (Figure 2). While the patient counts show a positive upward trend (an estimated 8.7% increase per year), this trend is not statistically significant at the $\alpha = 0.05$ level.

Table 2 Demographic Characteristics of Inpatients Identified by ICD-10 Code A31.1 (n = 103)

Features	N (%)	Adjusted 95% CI
Age		
Mean ± SD	52.11 ± 18.70	48.5–55.7
Gender		
Female	54/103 (52.43)	42.8–62.1
Male	49/103 (47.57)	37.9–57.2
Age group (years)		
0–20	7/103 (6.80)	3.1–13.6
20–40	16/103 (15.53)	9.7–23.9
40–60	38/103 (36.89)	28.2–46.5
60–80	39/103 (37.86)	29.1–47.5
> 80	3/103 (2.91)	0.6–8.6
BMI*		
Underweight	30/67 (44.78)	33.5–56.6
Normal	30/67 (44.78)	33.5–56.6
Overweight	4/67 (5.97)	1.39–14.8
Obesity	3/67 (4.48)	1.0–12.9

*BMI data were available for 67 of the 103 total cases.

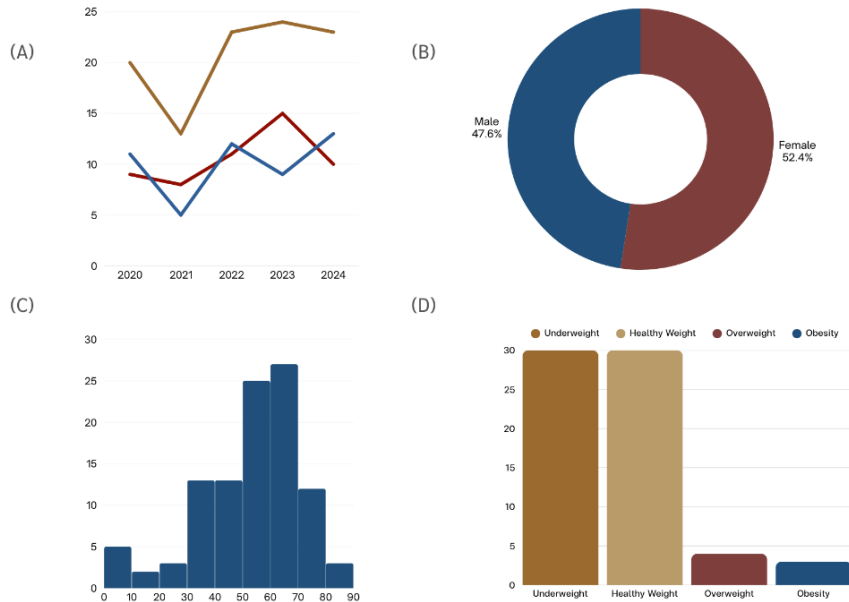


Figure 2 Demographic Profile of Patients with Cutaneous NTM Infection Admitted to Inpatient Departments in Thailand between 2020 and 2024. (A) illustrates the annual case trend over the five-year period; (B) presents the sex distribution of affected individuals; (C) displays the age distribution of the cohort; and (D) demonstrates the body mass index (BMI) distribution among patients for whom BMI data were available.

Occupation and environmental risk factors

Among the 103 patients admitted with cutaneous NTM infection (ICD-10 code A31.1), occupational information was available for 101 individuals. The majority were categorised as working in elementary occupations, with 75 cases (74.3%), which typically do not require specialised technical skills. The second most common category comprised skilled agricultural, forestry and fishery workers at 21 cases (20.8%). Other occupational groups, including managers, professionals, technicians, service and sales workers, and

craft and related trades workers, were represented by single cases in each category.

For the purpose of assessing environmental contact relevant to exposure risk, only skilled agricultural, forestry and fishery workers were classified as environmentally exposed. Accordingly, approximately one-fifth of patients in this cohort were considered to have occupational exposure to environmental sources potentially associated with NTM infection (Table 3 and Figure 3).

Table 3 Distribution of A31.1 Patients Across Occupational Classifications

Occupational classification	Cases
Managers	1
Professionals	1
Technicians and Associate Professionals	1
Clerical Support Workers	0
Service and Sales Workers	1
Skilled Agricultural, Forestry and Fishery Workers	21
Craft and Related Trades Workers	1
Plant and Machine Operators, and Assemblers	0
Elementary Occupations	75

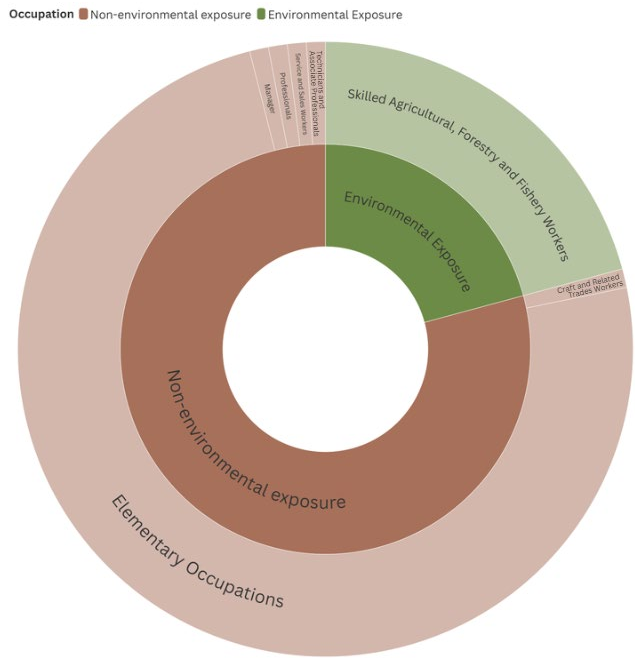


Figure 3 Distribution of Occupational Categories among 101 Patients with Cutaneous Nontuberculous Mycobacterial Infection in Thailand, 2020–2024.

Geographical distribution

Residential address records for all patients with A31.1-confirmed cutaneous NTM infection indicated that the largest proportion of cases originated from the Northeastern region, accounting for over half of the cohort, at 56.3% (95% CI: 46.7%–65.5%). The Central region contributed 22 cases (21.40%; 95% CI: 14.6%–30.2%), followed by comparable numbers of cases from the Southern (n = 9) and Northern (n = 10) regions. Three cases were reported from the Eastern region, and one case was identified from the Western region. The analysis of A31.1-confirmed cutaneous NTM infection cases across the N = 103 patient cohort revealed a highly non-uniform geographical distribution. The largest proportion of cases originated from the Northeastern region, accounting for 56.3% (n = 58) of the cohort (95% CI:

46.7%–65.5%). The Central region contributed 21.4% (n = 22; 95% CI: 14.6%–30.2%), followed by comparable numbers from the Southern (n = 9) and Northern (n = 10) regions. Only three cases were reported from the Eastern region, and one case was identified from the Western region. A chi-square goodness-of-fit test confirmed that the patient distribution across the six regions was statistically significant, indicating a non-uniform distribution ($X^2 = 132.28$, $df = 5$, $p < 0.001$). At the provincial level, Khon Kaen reported the highest number of cases (n = 15). Six cases each were identified in Surin and Bangkok. Nan province reported five cases. In addition, a total of 16 cases were recorded across four provinces in the Northeastern region—Maha Sarakham, Sakon Nakhon, Udon Thani, and Ubon Ratchathani—each contributing four cases (Figure 4).

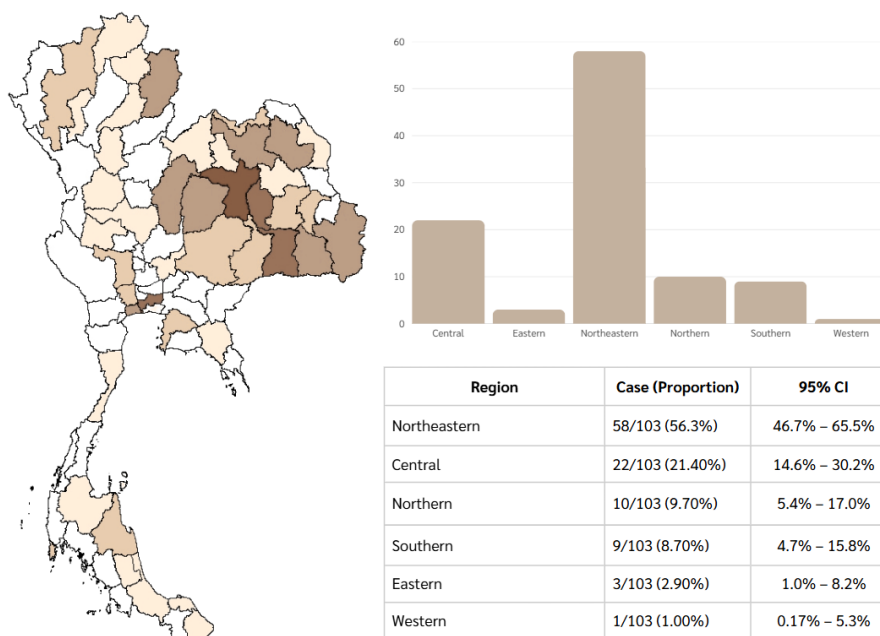


Figure 4 Geographical Distribution of Cutaneous NTM Patients across Inpatient Units. The Northeastern region recorded the highest number of diagnosed cases, followed by the Central region, while the Western region exhibited the lowest number of cases.

Therapeutic regimens and antibiotic prescribing patterns

Among the 103 patients diagnosed with cutaneous NTM, antibiotic prescribing patterns demonstrated a marked reliance on a limited number of therapeutic classes (Figure 5A). Azithromycin was the most frequently prescribed antibiotic (n = 39), followed by ethambutol (n = 37) and rifampicin (n = 34). Overall, macrolides, rifamycins, ethambutol, and fluoroquinolones constituted the principal therapeutic groups utilised in clinical management. Within the macrolide class, azithromycin was prescribed more frequently than clarithromycin, and rifampicin was the sole rifamycin employed in this cohort.

Levofloxacin was the most commonly administered fluoroquinolone, whereas ciprofloxacin, ofloxacin, and moxifloxacin were prescribed infrequently. Amikacin represented the only aminoglycoside used for cutaneous

NTM treatment, with no recorded use of streptomycin or tobramycin. Doxycycline, a tetracycline derivative, and meropenem from the carbapenem group were used at similarly low but notable frequencies, indicating their roles as secondary or adjunctive agents. Cephalosporins, including cefazolin, ceftriaxone, and ceftazidime, were rarely prescribed. A small proportion of patients received piperacillin-tazobactam, while imipenem-cilastatin demonstrated a slightly higher frequency of use. Co-trimoxazole was prescribed at a comparable rate to amikacin. Isoniazid, primarily used in tuberculosis therapy, was prescribed at approximately half the frequency of azithromycin among patients with cutaneous NTM.

In clinical practice, antibiotics for cutaneous NTM were predominantly prescribed in combination rather than as monotherapy (Figure 5B). Rifampicin and ethambutol frequently co-occurred with azithromycin, amikacin, and

levofloxacin, consistent with commonly used combination regimens for NTM treatment²². Ethambutol and macrolides were commonly incorporated into triple or quadruple combination regimens, forming the core of therapeutic approaches. In contrast, meropenem and amoxicillin-clavulanic acid exhibited minimal network connectivity,

indicating limited roles in routine co-prescription patterns. Collectively, the network structure illustrates that treatment regimens in this cohort were not random but were characterised by consistent, centralised combinations, predominantly centred on macrolide-ethambutol-rifampicin-based regimens.

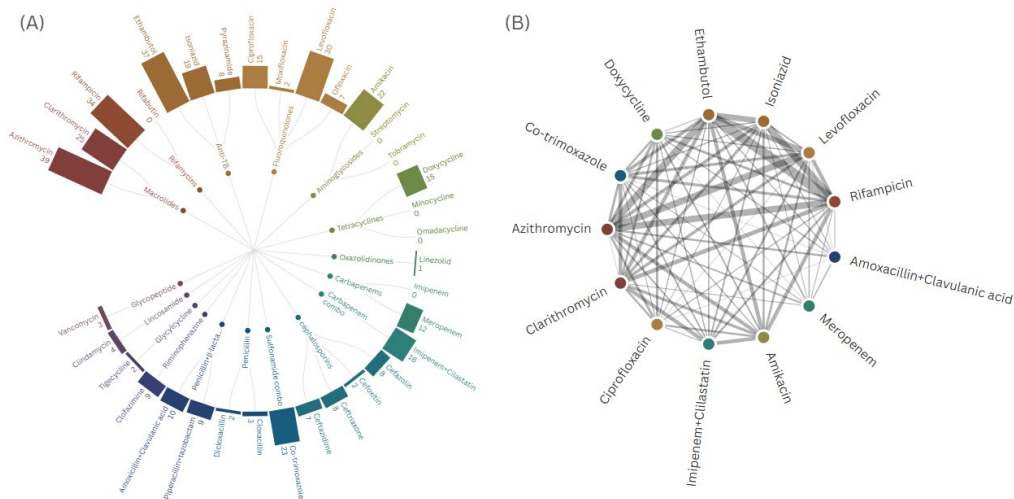


Figure 5 Antibiotic Prescribing Patterns in Cutaneous NTM Management. (A) Frequency of individual antibiotic prescriptions among 103 patients. (B) Co-prescription network demonstrating antibiotic combinations used in patients, highlighting core multidrug regimens.

Clinical features

Analysis of chief complaint records showed that the reported incubation period among inpatients diagnosed with A31.1 varied widely, ranging from a few days to many years. However, this incubation period was rarely reported and therefore could not be meaningfully evaluated. The clinical presentations were grouped into eight symptom categories. Several entries also contained non-clinical information such as payment status, referral details, and ward assignment. Although there was no standardised format for documenting chief complaints and symptom descriptions were not consistently available across all

cutaneous NTM cases, the most frequently reported clinical feature was systemic malaise, typically characterised by fatigue, fever, and loss of appetite. Dermatological or cutaneous manifestations were also common presentations. Pain-related complaints and respiratory symptoms were observed at comparable frequencies, whereas chronic comorbidities were reported less often. A minority of patients received clinical interventions such as surgical procedures or blood transfusions. Gastrointestinal symptoms and tuberculosis-related features were noted only in a small subset of cases (Table 4 and Figure 6).

Table 4 Free-Text Chief Complaints Harmonised into Predefined symptom categories

Symptom categories	Terms
Systemic malaise	Fatigue, loss of appetite, weight loss, chills, shivering, fever, chills
Pain	Pain, soreness, tenderness, chest tightness (if non-respiratory)
Respiratory symptoms	Dyspnoea, shortness of breath, cough, sputum, chest tightness (if respiratory context), runny nose, nasal congestion
Neurovestibular symptoms	Insomnia, drowsiness, syncope, dizziness, altered consciousness
Cutaneous lesions	Wounds, ulcers, necrotic tissue, chronic wounds, panniculitis, swelling, nodules
Gastrointestinal symptoms	Dyspepsia, nausea, vomiting, abdominal discomfort
Tuberculosis	Tuberculous lymphadenitis, disseminated TB
Other chronic diseases	Thalassaemia, RA, leprosy, CGD, peripheral neuropathy

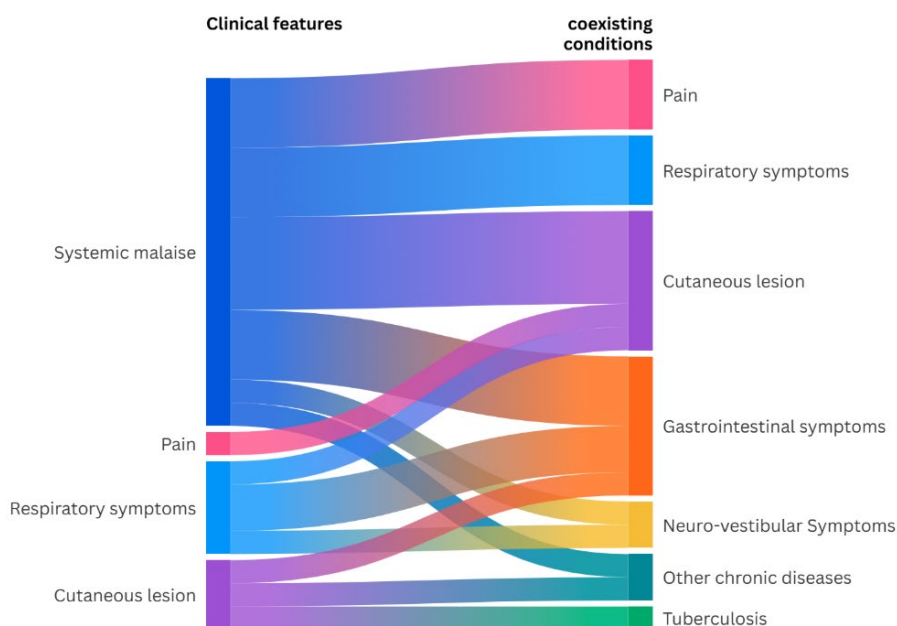


Figure 6 Sankey Diagram Illustrating the Distribution of Clinical Features Documented in Chief Complaints and Their Corresponding Clinical Categories. This visualisation highlights the heterogeneity of symptom profiles and the overlap between systemic presentations and specific organ-related manifestations in patients with cutaneous NTM.

Discussion

Interpretation of these findings must be considered within the limitations of the available data. Fragmentation of Thailand's health information systems and limited familiarity with complex coding frameworks among healthcare personnel may have contributed to data inconsistencies and patient misclassification. Reliance on the ICD-10 code A31.1, which lacks species-level specificity, further restricts species-based epidemiological and antimicrobial analyses.

Exposure assessment was also limited. Occupational data were used as a proxy for environmental exposure, but detailed exposure histories and incubation periods were rarely recorded, and chief complaint notes were highly variable and largely non-standardised. Accordingly, symptom patterns in this study should be interpreted as indicative rather than definitive. A temporary decline in pulmonary and cutaneous NTM cases (A31.1) was observed in 2021, likely reflecting reduced healthcare access and diagnostic throughput during the COVID-19 pandemic²³. Although elementary occupations commonly involve soil and water exposure, the available data primarily included retirees, sales workers, clerical staff, and students; thus, this category was excluded from environmental exposure analysis²⁴.

In terms of clinical presentation, cutaneous and dermatological manifestations were recorded less frequently than systemic malaise in the chief complaints. This pattern does not necessarily indicate that cutaneous NTM commonly progresses to systemic involvement. It is possible that clinicians, recognising A31.1 as a code for skin-related infection, may have prioritised documenting additional systemic symptoms instead of dermatological findings. Conversely, the predominance of non-cutaneous presentations may reflect miscoding or inclusion of cases that did not truly represent cutaneous NTM. This possibility

highlights the need for careful diagnostic classification and more stringent recording of primary skin-related features in suspected NTM cases.

Geographical analysis showed the highest case count in Bangkok and the greatest regional burden in the Northeast, suggesting diagnostic accessibility as a major determinant of case detection. Enhanced diagnostic infrastructure in the capital likely accounts for improved case discovery, whereas the higher incidence in the Northeast may signify true endemic circulation concealed by diagnostic gaps in other regions. Finally, therapeutic assessment was limited to antibiotic co-prescription at the first recorded episode within a given year, precluding evaluation of subsequent treatment modification or response.

Conclusions

This study utilised the Thai national health database (43 files) to characterise the demographic and therapeutic profiles of inpatients coded with ICD-10 A31.1. The condition was more common among females (53%), with a mean age of 52 years, and was predominantly reported among individuals with normal or underweight BMI. Approximately one-fifth of cases worked in agriculture, forestry or fisheries, suggesting that environmental contact is a key occupational exposure pathway. Khon Kaen exhibited the highest case count at the provincial level, while the Northeast constituted the region with the greatest overall burden.

The predominance of systemic malaise over classic dermatological presentations indicates a limitation of relying solely on A31.1 coding for case identification, raising the possibility of misclassification or inclusion of systemic NTM infections. Treatment patterns demonstrated routine implementation of multidrug regimens consistent with international recommendations for NTM management. Ethambutol and rifampicin were most frequently used

as first-line agents, typically alongside azithromycin, while broader combination therapy often incorporated levofloxacin and amikacin.

Future research should integrate microbiological confirmation and species identification to strengthen clinical interpretation. Further pharmaco-epidemiological analysis encompassing all concomitant medications, not only antibiotics, would lead to a more comprehensive understanding of therapeutic practice in cutaneous NTM. Strengthening ICD-10 coding competency, particularly for A31-related classifications, is essential to enhance data accuracy and uphold the quality of national health information.

Ethical statement

The study was approved by the Institutional Human Research Ethics Committee of Ministry of Public Health, Thailand (protocol code Ref.no.20/2567; approval date: January 9, 2025). All data were anonymised and encrypted prior to analysis to ensure the protection of patient confidentiality. In accordance with the retrospective nature of the study, individual informed consent was not required.

Acknowledgements

This study was supported by the National Science and Research Fund (NSRF) through the Program Management Unit for Human Resources & Institutional Development, Research and Innovation (PMU-B) [Grant No. B50G670096]. We express our deep appreciation to Prof. Taane G. Clark (London School of Hygiene and Tropical Medicine) for his constructive review and guidance, and to Ms Kanisorn Larpadisorn, a registered pharmacist, for her valuable contributions to antibiotic classification and interpretation.

References

- Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175(4):367-416. doi:10.1164/rccm.200604-571ST.
- Koh WJ. Nontuberculous mycobacteria-overview. *Microbiol Spectr* 2017;5(1):10.1128/microbiolspec.tnmi7-0024-2016. doi:10.1128/microbiolspec.TNMI7-0024-2016.
- Falkinham JO 3rd. Environmental sources of nontuberculous mycobacteria. *Clin Chest Med* 2015;36(1):35-41. doi:10.1016/j.ccm.2014.10.003.
- Bi S, Hu FS, Yu HY, et al. Nontuberculous mycobacterial osteomyelitis. *Infect Dis (Lond)* 2015;47(10):673-85. doi:10.3109/23744235.2015.1040445.
- Fowler SJ, French J, Screaton NJ, et al. Nontuberculous mycobacteria in bronchiectasis: Prevalence and patient characteristics. *Eur Respir J* 2006;28(6):1204-10. doi:10.1183/09031936.06.00149805.
- Adjemian J, Olivier KN, Seitz AE, et al. Spatial clusters of nontuberculous mycobacterial lung disease in the United States. *Am J Respir Crit Care Med* 2012;186(6):553-8. doi:10.1164/rccm.201205-0913OC.
- Chan WS, Tee SI, Chandran NS, et al. Two episodes of cutaneous non-tuberculous mycobacterial infection in a patient with psoriasis. *Dermatol Reports* 2015;7(2):5712. doi:10.4081/dr.2015.5712.
- Jhun BW, Moon SM, Jeon K, et al. Prognostic factors associated with long-term mortality in 1445 patients with nontuberculous mycobacterial pulmonary disease: A 15-year follow-up study. *Eur Respir J* 2020;55(1):1900798. doi:10.1183/13993003.00798-2019.
- Alkan Bilik O, Ozcan N, Selimoglu Sen H, et al. Species diversity and clinical relevance of nontuberculous mycobacterium isolated from pulmonary and extrapulmonary samples in southeastern Turkey, 2014 to 2023: A retrospective cross-sectional study. *Medicine (Baltimore)* 2025;104(29):e43415. doi:10.1097/MD.00000000000043415.
- Pennington KM, Vu A, Challener D, et al. Approach to the diagnosis and treatment of non-tuberculous mycobacterial disease. *J Clin Tuberc Other Mycobact Dis* 2021;24:100244. doi:10.1016/j.jctube.2021.100244.
- van Ingen J. Diagnosis of nontuberculous mycobacterial infections. *Semin Respir Crit Care Med* 2013;34(1):103-9. doi:10.1055/s-0033-1333569.
- Conyers LE, Saunders BM. Treatment for non-tuberculous mycobacteria: Challenges and prospects. *Front Microbiol* 2024;15:1394220.
- Lee BY, Kim S, Hong Y, et al. Risk factors for recurrence after successful treatment of *Mycobacterium avium* complex lung disease. *Antimicrob Agents Chemother* 2015;59(6):2972-7. doi:10.1128/AAC.04577-14.
- Koh WJ, Moon SM, Kim SY, et al. Outcomes of *Mycobacterium avium* complex lung disease based on clinical phenotype. *Eur Respir J* 2017;50(3):1602503. doi:10.1183/13993003.02503-2016.
- Prevots DR, Marshall JE, Wagner D, et al. Global epidemiology of nontuberculous mycobacterial pulmonary disease: A review. *Clin Chest Med* 2023;44(4):675-721. doi:10.1016/j.ccm.2023.08.012.
- Dahl VN, Molhave M, Floe A, et al. Global trends of pulmonary infections with nontuberculous mycobacteria: A systematic review. *Int J Infect Dis* 2022;125:120-131. doi:10.1016/j.ijid.2022.10.013.
- Chirasuthat P, Triyangkulsri K, Rutnin S, et al. Cutaneous nontuberculous mycobacterial infection in Thailand: A 7-year retrospective review. *Medicine (Baltimore)* 2020;99(10):e19355. doi:10.1097/MD.00000000000019355.
- Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 2010;182(7):970-6. doi:10.1164/rccm.201002-0310OC.
- Winthrop KL, Marras TK, Adjemian J, et al. Incidence and prevalence of nontuberculous mycobacterial lung disease in a Large U.S. Managed Care Health Plan, 2008-2015. *Ann Am Thorac Soc* 2020;17(2):178-85. doi:10.1513/AnnalsATS.201804-236OC.
- Blakney RA, Ricotta EE, Frankland TB, et al. Incidence of nontuberculous mycobacterial pulmonary infection, by Ethnic Group, Hawaii, USA, 2005-2019. *Emerg Infect Dis* 2022;28(8):1543-50. doi:10.3201/eid2808.212375.
- Kang JY, Han K, Kim MK. Severity of underweight affects the development of nontuberculous mycobacterial pulmonary disease; a nationwide longitudinal study. *Sci Rep* 2022;12(1):17180. doi:10.1038/s41598-022-21511-x.
- Daley CL, Iaccarino JM, Lange C, et al. Treatment of nontuberculous mycobacterial pulmonary disease: An official ATS/ERS/ESCMID/IDSA clinical practice guideline. *Clin Infect Dis* 2020;71(4):e1-36. doi:10.1093/cid/ciaa241.

23. Jeong Y, Min J. Impact of COVID-19 pandemic on tuberculosis preventive services and their post-pandemic recovery strategies: A rapid review of literature. *J Korean Med Sci* 2023;38(5):e43. doi:10.3346/jkms.2023.38.e43.
24. Mercaldo RA, Marshall JE, Cangelosi GA, et al. Environmental risk of nontuberculous mycobacterial infection: Strategies for advancing methodology. *Tuberculosis (Edinb)* 2023;139:102305. doi:10.1016/j.tube.2023.102305.

ORIGINAL ARTICLE

Trend Analysis and Factors Associated with the Results of Rifampicin-Resistant Tuberculosis Testing Using the Gene Xpert MTB/RIF in Patients at Pathum Thani Hospital

Puthapach Chailuang

Division of Medical Technology and Clinical Pathology, Pathum Thani Hospital

KEYWORDS

drug-resistant tuberculosis; cycle threshold value; rifampicin; gene Xpert; trends

CORRESPONDING AUTHOR

Puthapach Chailuang

Division of Medical Technology and Clinical Pathology, Pathum Thani Hospital
7 Pathum Thani-Lat Lum Kao Road, Bang Prok Sub-district, Mueang District, Pathum Thani 12000, Thailand
E-mail: puthapach@gmail.com

Received: March 30, 2026
Revised: August 22, 2026
Accepted: August 27, 2026

ABSTRACT

This study aimed to analyse temporal trends of *Mycobacterium tuberculosis* (MTB) detection and identify factors associated with rifampicin resistance (RR-TB) using the Gene Xpert MTB/RIF system among patients at Pathum Thani Hospital. A retrospective analytical study was conducted using secondary diagnostic and clinical data from 6,059 patients tested between January 2022 and December 2024. Statistical analyses included descriptive statistics, the chi-square test for trend, chi-square test of independence, independent t-test, and multivariable logistic regression analysis. Overall, MTB was detected in 1,244 cases (20.5%), with annual detection rates of 25.2% (313/1,241) in 2022, 27.3% (340/1,246) in 2023, and 16.5% (591/3,572) in 2024, demonstrating a statistically significant trend in MTB detection across the study period ($p < 0.001$). Among MTB-positive cases, rifampicin resistance was identified in 236 patients (19.0%), with annual proportions of 20.1% (63/313) in 2022, 22.4% (76/340) in 2023, and 16.4% (97/591) in 2024 ($p = 0.042$). Multivariable logistic regression revealed that a prior history of tuberculosis treatment was the strongest independent predictor of rifampicin resistance (adjusted OR = 3.10; 95% CI: 2.33–4.12; $p < 0.001$). Other factors significantly associated with resistance included age ≥ 40 years (adjusted OR = 1.56; 95% CI: 1.12–2.17; $p < 0.05$), positive acid-fast bacilli (AFB) smear status (adjusted OR = 1.71; 95% CI: 1.23–2.38; $p < 0.05$), and abnormal chest radiographic findings (adjusted OR = 2.77; 95% CI: 1.50–5.10; $p < 0.01$). Male sex was not significantly associated with resistance (adjusted OR = 1.20; 95% CI: 0.87–1.66; $p = 0.08$). The rifampicin-resistant group exhibited significantly lower mean cycle threshold (Ct) values compared to the non-resistant group (20.1 ± 3.9 vs. 23.0 ± 4.7 ; $p < 0.01$), indicating a significantly higher bacillary burden. These findings highlight the critical role of the Gene Xpert system in facilitating rapid detection and risk stratification to support tuberculosis control efforts at the provincial level.

Introduction

Tuberculosis (TB) remains a major global public health threat, caused by the airborne pathogen *Mycobacterium tuberculosis* (MTB). According to the World Health Organization (WHO), Thailand is classified among the 30 high TB-burden countries globally, facing severe challenges regarding drug-resistant tuberculosis strains^{1,2}. The WHO's "End TB Strategy" aims to reduce TB incidence by 90% and TB deaths by 95% by 2035³. However, diagnostic delays, incomplete case notification, and suboptimal treatment adherence continue to undermine disease control efforts worldwide, particularly in developing countries and rapidly urbanizing provinces^{1,4}.

Conventional diagnostic methodologies, including acid-fast bacilli (AFB) smear microscopy and mycobacterial culture, have served as traditional mainstays in clinical laboratories. Nevertheless, conventional microscopy suffers

from limited diagnostic sensitivity—ranging between 40% and 60%—and frequently yields false-negative results in paucibacillary specimens, pediatric cases, patients living with HIV, and extrapulmonary presentations such as tuberculous meningitis^{4,7}. Conversely, while mycobacterial culture remains the gold standard for diagnosis and phenotypic drug susceptibility testing (DST), its turnaround time of 2 to 8 weeks substantially delays clinical decision-making, thereby increasing the risk of ongoing community transmission of multidrug-resistant tuberculosis (MDR-TB)^{5,8}.

To overcome these traditional barriers, automated real-time nucleic acid amplification technologies, specifically the Gene Xpert MTB/RIF system, have been integrated into routine public health workflows. The Gene Xpert system allows simultaneous, rapid identification of MTB DNA and detection of mutations in the *rpoB* gene conferring rifampicin resistance within approximately two hours^{8,9}.

Because rifampicin resistance serves as a reliable surrogate marker for MDR-TB, early molecular detection enables immediate triage and initiation of appropriate second-line therapeutic regimens in accordance with national tuberculosis control guidelines⁹⁻¹¹. Furthermore, advanced molecular iterations such as the Xpert Ultra assay have further enhanced diagnostic sensitivity in paucibacillary and extrapulmonary specimens^{12,13}. Consequently, the WHO strongly recommends Gene Xpert as the initial diagnostic test for all individuals presenting with signs and symptoms of tuberculosis^{1,12}.

In addition to qualitative detection, the Gene Xpert system generates semi-quantitative automated data represented by cycle threshold (Ct) values. The Ct value corresponds inversely to the log concentration of target DNA in the sample; hence, lower Ct values denote higher bacillary loads^{14,15}. Recent epidemiological and clinical studies indicate that high bacillary burden is strongly correlated with increased infectiousness, delayed sputum conversion, and severe pulmonary tissue destruction observed on chest radiography^{15,16}. Furthermore, quantifying bacterial load via Ct values provides valuable surrogate insights into disease severity and transmission dynamics¹⁴⁻¹⁷.

Pathum Thani Province, situated in the central region of Thailand, is characterized by dense urbanization, rapid industrial growth, and high population mobility. Pathum Thani Hospital operates as the primary provincial tertiary referral center, receiving complex clinical cases and suspected drug-resistant TB referrals from local community hospitals. Establishing long-term trends of MTB detection, monitoring rifampicin resistance rates, and evaluating associated clinical predictors within this high-density population are critical for refining diagnostic protocols and optimizing regional tuberculosis control programs^{2,17,18}. Therefore, this retrospective study aimed to analyze the temporal trends of MTB detection and identify independent factors associated with rifampicin resistance using Gene Xpert data collected at Pathum Thani Hospital between 2022 and 2024.

Materials and Methods

Study Design

This study was designed as a retrospective analytical study using secondary laboratory and electronic medical records. The objective was to evaluate three-year temporal trends of MTB detection and analyze demographic, microbiological, and radiological factors associated with rifampicin resistance among patients tested via the Gene Xpert MTB/RIF system.

Study Site and Population

The study was conducted at the Division of Medical Technology and Clinical Pathology, Pathum Thani Hospital, Pathum Thani Province, Thailand. The target population comprised all patients presenting with clinical suspicion of pulmonary or extrapulmonary tuberculosis who underwent Gene Xpert MTB/RIF testing between January 1, 2022, and December 31, 2024.

Eligibility Criteria

Inclusion Criteria: All patient records with completed Gene Xpert MTB/RIF diagnostic results performed during the study period, accompanied by valid demographic data (age, sex, and documented history of prior TB treatment), AFB smear results, and chest radiographic reports.

Exclusion Criteria: Duplicated laboratory records, indeterminate or invalid Gene Xpert test results, and cases with missing essential demographic or clinical variables required for multivariable analysis.

Data Collection and Variables

Secondary data were systematically extracted from the hospital's Laboratory Information System (LIS) and electronic medical records using a standardized data extraction protocol. The collected variables included:

1. *Demographic & Clinical Characteristics:* Age, sex, and history of prior tuberculosis treatment (categorized as new case vs. previously treated case). Age was dichotomized at ≥ 40 years based on the sample distribution and clinical relevance.

2. *Microbiological & Molecular Data:* AFB smear microscopy status (positive vs. negative), Gene Xpert MTB detection status, rifampicin resistance status (resistant vs. sensitive), and Gene Xpert Ct values.

3. *Radiological Findings:* Chest X-ray reports interpreted by board-certified radiologists, categorized as normal or abnormal (presence of infiltrates, cavities, nodular opacities, or pleural effusion).

Statistical Analysis

Data were verified, de-identified, and analyzed using standard statistical software. Categorical variables were presented as absolute frequencies and percentages. Continuous variables (Ct values) were reported as mean standard deviation (SD). Temporal trends in MTB detection rates over the three-year study period were evaluated using the chi-square test for trend. Annual variations in rifampicin resistance proportions were tested using the chi-square test of independence.

Univariable and multivariable logistic regression analyses were conducted to evaluate factors associated with rifampicin resistance. Crude Odds Ratios (OR) and Adjusted Odds Ratios (adjusted OR) with 95% Confidence Intervals (95% CI) were calculated. Differences in mean Ct values across resistance status, AFB smear results, and chest radiographic findings were compared using the independent t-test. Statistical significance was established at two-tailed $p < 0.05$.

Results

Between 2022 and 2024, a total of 6,059 patients suspected of having tuberculosis were screened using the Gene Xpert system at Pathum Thani Hospital. *Mycobacterium tuberculosis* (MTB) was detected in 1,244 cases (20.5%), with annual detection rates of 25.2% (313/1,241) in 2022, 27.3% (340/1,246) in 2023, and 16.5% (591/3,572) in 2024. Among the MTB-positive cases, rifampicin resistance (RR-MTB) was identified in 236 patients (19.0%), with annual proportions of 20.1% in 2022, 22.4% in 2023, and 16.4% in 2024 (Table 1).

Multivariable logistic regression analysis of demographic characteristics and clinical data identified key factors associated with rifampicin resistance. A prior history of tuberculosis treatment was the strongest independent predictor of resistance (adjusted OR = 3.10; 95% CI: 2.33–4.12; $p < 0.001$). Other factors significantly associated with resistance included age ≥ 40 years (adjusted OR = 1.56; 95% CI: 1.12–2.17; $p < 0.05$), AFB smear positivity (adjusted OR = 1.71; 95% CI: 1.23–2.38; $p < 0.05$), and abnormal chest radiographic findings (adjusted OR = 2.77; 95% CI: 1.50–5.10;

$p<0.01$). Male sex showed a trend toward higher resistance but did not achieve statistical significance (adjusted OR = 1.20; 95% CI: 0.87–1.66; $p=0.08$) (Table 2).

Detailed annual analysis confirmed a consistent association between prior tuberculosis treatment history and rifampicin resistance across all three study years (Table 3). Overall, patients with a history of prior TB treatment demonstrated a significantly higher proportion of rifampicin resistance (35.7%, 122/342) compared to newly diagnosed cases (12.6%, 114/902), representing a 3.8-fold increase in unadjusted odds (unadjusted OR = 3.83; 95% CI: 2.85–5.15; $p<0.001$) (Table 3).

Analysis of cycle threshold (Ct) values among MTB-positive cases revealed significant differences based on resistance status, microbiological findings, and radiological

presentation. Patients with rifampicin-resistant TB exhibited significantly lower mean Ct values (20.1 ± 3.9) compared to non-resistant cases (23.0 ± 4.7 ; $p<0.01$). Similarly, significantly lower Ct values were observed in patients with positive AFB smears (19.8 ± 4.1 vs. 24.2 ± 4.8 for AFB-negative; $p<0.01$) and those with abnormal chest X-ray findings (21.9 ± 4.4 vs. 25.1 ± 4.9 for normal chest X-ray; $p<0.01$) (Table 4).

Evaluation of diagnostic concordance showed that AFB smear positivity was strongly associated with both MTB detection and rifampicin resistance. Overall, 1,068 patients (17.6%) were AFB smear-positive. Smear-positive status was significantly associated with higher MTB detection rates and rifampicin resistance compared to smear-negative status ($p<0.001$) (Table 5).

Table 1 Summary of MTB detected and rifampicin-resistant cases classified by year (n=6,059)

Year (B.E.)	Total patients tested (n)	MTB detected (n, %)	Rifampicin resistance (n, % of MTB+)
2022 (2565)	1,241	313 (25.2)	63 (20.1)
2023 (2566)	1,246	340 (27.3)	76 (22.4)
2024 (2567)	3,572	591 (16.5)	97 (16.4)
Total	6,059	1,244 (20.5)	236 (19.0)

Footnote: Chi-square test for trend was used to analyze the trend of MTB detection across years ($p<0.001$). Chi-square test of independence was used to analyze the annual variation of rifampicin resistance proportions among MTB-positive cases ($p=0.042$).

Table 2 Analysis of demographic characteristics and clinical factors associated with rifampicin resistance among MTB-positive cases (n=1,244)

Demographic characteristics & clinical data	Total (n)	Resistant (n, %)	Sensitive (n, %)	Adjusted OR (95% CI)	p-value
Previous TB treatment history					
Yes	342	122 (35.7)	220 (64.3)	3.10 (2.33–4.12)	< 0.001
No (New case)	902	114 (12.6)	805 (89.2)	1.00 (Reference)	
Age group					
≥ 40 years	864	179 (20.7)	685 (79.3)	1.56 (1.12–2.17)	< 0.05
< 40 years	380	58 (15.3)	322 (84.7)	1.00 (Reference)	
Sex					
Male	908	179 (19.7)	729 (80.3)	1.20 (0.87–1.66)	0.08
Female	336	57 (17.0)	279 (83.0)	1.00 (Reference)	
AFB smear result					
Positive	884	185 (20.9)	699 (79.1)	1.71 (1.23–2.38)	< 0.05
Negative	360	51 (14.2)	309 (85.8)	1.00 (Reference)	
Chest X-ray finding					
Abnormal	1,102	224 (20.3)	878 (79.7)	2.77 (1.50–5.10)	< 0.01
Normal	142	12 (8.5)	130 (91.5)	1.00 (Reference)	

Footnote: Multivariable logistic regression analysis was used to determine independent factors associated with rifampicin resistance. Adjusted Odds Ratios (OR) 1.00 (Reference) and 95% Confidence Intervals (CI) are reported.

Table 3 Association between history of tuberculosis treatment and rifampicin resistance classified by year

Year (B.E.)	Previously treated (N)	Rif + (n, %)	Not previously treated (N)	Rif + (n, %)
2022 (2565)	87	28 (32.2)	226	35 (15.5)
2023 (2566)	95	52 (54.7)	245	24 (9.8)
2024 (2567)	160	42 (26.3)	431	38 (8.8)
Total	342	122 (35.7)	902	114 (12.6)

Footnote: Chi-square test of independence was used for statistical analysis. 3-year combined unadjusted Odds Ratio (OR) = **3.83** (95% CI: 2.85–5.15), $p < 0.001$. Patients with a prior history of tuberculosis treatment were **3.8 times** more likely to have rifampicin resistance compared to non-previously treated patients.

Table 4 Mean Cycle Threshold (Ct) values in MTB-positive patient subgroups (n=1,244)

Patient subgroup	Mean Ct value (Mean $\pm$ SD)	<i>p</i> -value
All MTB-positive cases		
Overall	22.4 $\pm$ 4.6	
Rifampicin resistance		
Resistant	20.1 $\pm$ 3.9	< 0.01
Non-resistant	23.0 $\pm$ 4.7	
AFB smear status		
Positive	19.8 $\pm$ 4.1	< 0.01
Negative	24.2 $\pm$ 4.8	
Chest X-ray finding		
Abnormal	21.9 $\pm$ 4.4	< 0.01
Normal	25.1 $\pm$ 4.9	

Footnote: Independent t-test was used for comparison of mean Ct values between groups. Lower Ct values (indicating higher bacillary burden) were significantly associated with rifampicin resistance, positive AFB smear results, and abnormal chest X-ray findings ($p < 0.01$).

Table 5 Distribution of AFB smear positivity and Gene Xpert results by year (2022–2024) (n=6,059)

Year (B.E.)	Total tested (n)	AFB-positive (n, %)	MTB detected (n, % of Total)	Rifampicin-resistant (n, % of MTB+)
2022 (2565)	1,241	268 (21.6)	313 (25.2)	63 (20.1)
2023 (2566)	1,246	282 (22.6)	340 (27.3)	76 (22.4)
2024 (2567)	3,572	518 (14.5)	591 (16.5)	97 (16.4)
Total	6,059	1,068 (17.6)	1,244 (20.5)	236 (19.0)

Footnote: Chi-square test of independence was used for the analysis. AFB smear positivity was observed in 1,068 patients (17.6%). AFB-positive smear results were significantly associated with both MTB detection ($p < 0.001$) and rifampicin resistance ($p < 0.001$), supporting strong diagnostic concordance between microbiological microscopy and molecular assays.

Discussion

This retrospective analytical study provides important insights into the temporal trends of *Mycobacterium tuberculosis* (MTB) detection and factors associated with rifampicin resistance at Pathum Thani Hospital over a three-year period. A key observation in this study was the substantial surge in testing volume in 2024 (n=3,572), which nearly tripled the annual volume of previous years. Concurrently, the overall positivity rate in 2024 dropped to 16.5%, compared to 25.2% in 2022 and 27.3% in 2023. This shift reflects expanded screening protocols at Pathum Thani Hospital, incorporating Gene Xpert testing earlier in routine

diagnostic algorithms for broader patient populations rather than limiting testing strictly to high-risk or smear-positive individuals. Although the overall detection percentage decreased in 2024 due to a larger denominator, the absolute number of confirmed MTB cases reached its highest peak (n=591), demonstrating enhanced case finding capacity at the hospital level.

Regarding drug resistance, the overall proportion of rifampicin resistance among MTB-positive cases was 19.0%, with annual proportions ranging from 16.4% to 22.4%. This rate is higher than reported national averages for new TB cases in Thailand², largely because Pathum

Thani Hospital functions as a provincial referral center that routinely receives refractory, recurrent, and complex cases from surrounding secondary healthcare facilities. However, when expanding testing to lower-risk populations in 2024, the proportion of resistant cases among positive detections showed a slight decline to 16.4%. This trend suggests that broader screening successfully captures a higher proportion of drug-susceptible cases at earlier disease stages.

In multivariable logistic regression analysis, a prior history of tuberculosis treatment stood out as the most powerful independent risk factor for rifampicin resistance (adjusted OR = 3.10; 95% CI: 2.33–4.12; $p < 0.001$). This finding aligns with extensive global and national literature establishing prior anti-TB therapy as the primary driver of acquired drug resistance^{1,9,18}. Interrupted, incomplete, or inadequate treatment regimens create selective pressure that favors the survival and proliferation of resistant mutant strains¹⁰. Detailed annual breakdown in Table 3 further confirmed that previously treated patients consistently exhibited high resistance rates (ranging from 26.3% to 54.7% annually). This highlights the necessity of prioritizing previously treated patients for immediate molecular resistance screening and directly observed therapy (DOT) in accordance with national tuberculosis control guidelines^{2,11,18}.

Age ≥ 40 years was also significantly associated with rifampicin resistance (adjusted OR = 1.56; 95% CI: 1.12–2.17). Older individuals may have higher cumulative exposure to TB over time or hidden prior treatment episodes that were unrecorded¹⁸. Furthermore, clinical indicators of disease burden, including positive AFB smears (adjusted OR = 1.71) and abnormal chest radiographs (adjusted OR = 2.77), were significantly linked to rifampicin resistance. These observations indicate that drug-resistant TB cases frequently present with more advanced anatomical lesions and higher mycobacterial loads at the time of diagnosis^{14,18}.

The semi-quantitative cycle threshold (Ct) values generated by Gene Xpert provided additional quantitative confirmation of bacterial load. Patients with rifampicin-resistant TB exhibited significantly lower mean Ct values (20.1 ± 3.9) compared to non-resistant cases (23.0 ± 4.7 ; $p < 0.01$), indicating a higher bacillary load. High bacterial burden in resistant cases likely reflects delayed diagnosis, ongoing bacterial replication under ineffective therapy, or extensive pulmonary cavitation^{14–16}. Low Ct values were also significantly correlated with AFB smear positivity (19.8 ± 4.1) and abnormal chest X-rays (21.9 ± 4.4). While Ct values cannot serve as a standalone diagnostic tool for drug resistance, incorporating Ct value reporting into routine laboratory feedback can help clinicians identify highly infectious patients who require immediate respiratory isolation and prioritized follow-up^{14–17}.

Several limitations should be considered when interpreting these findings

1. Retrospective Design: The study relied on secondary electronic health records, which inherently introduces the possibility of missing data or selection bias.
2. Unmeasured Confounders: Important clinical variables, including HIV status, diabetes mellitus, pediatric cases, extrapulmonary presentations (e.g., tuberculous meningitis), and the use of higher-sensitivity assays like Xpert Ultra, were not consistently recorded in the secondary dataset and could not be controlled for in the multivariable model^{6,7,13}.

3. Lack of Direct Clinical Outcomes: As a diagnostic and epidemiological evaluation, the study did not follow patients prospectively to measure long-term treatment outcomes, mortality, or transmission rates.

Conclusions

This study demonstrates that the Gene Xpert system is a vital molecular diagnostic tool for the rapid identification of *Mycobacterium tuberculosis* and rifampicin resistance in a high-volume provincial hospital setting. Prior tuberculosis treatment history represents the strongest predictor of rifampicin resistance, alongside age ≥ 40 years, AFB smear positivity, and abnormal chest radiographs. Additionally, lower Ct values among resistant cases highlight a higher bacillary burden and transmission risk. Strengthening universal access to molecular screening, improving treatment adherence tracking, and integrating laboratory Ct values into clinical risk management are essential strategies for curbing drug-resistant tuberculosis in Pathum Thani Province^{2,17}.

Ethical statement

This study was conducted in accordance with the Declaration of Helsinki and was approved by the Human Research Ethics Committee of Pathum Thani Hospital (Approval No. EC-PTH A147-68; approval date: November 21, 2025). Patient confidentiality was strictly protected using secondary, fully anonymized, and de-identified laboratory datasets. Informed consent was waived by the ethics committee due to the observational retrospective nature of the study.

References

1. World Health Organization. Global tuberculosis report 2023. Geneva: World Health Organization; 2023. Available from: <https://www.who.int/publications/i/item/9789240083851>.
2. Division of Tuberculosis, Department of Disease Control. Tuberculosis situation report in Thailand 2022. Bangkok: Ministry of Public Health; 2023. Available from: <https://www.tbthailand.org>
3. Uplekar M, Weil D, Lonnroth K, et al. WHO's new End TB Strategy. *Lancet* 2015;385(9979):1799–801. doi:10.1016/S0140-6736(15)60570-0.
4. Steingart KR, Schiller I, Horne DJ, et al. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2014;2014(1):CD009593. doi:10.1002/14651858.CD009593.pub3.
5. Theron G, Peter J, van Zyl-Smit R, et al. Evaluation of the Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *Am J Respir Crit Care Med* 2011;184(1):132–40. doi:10.1164/rccm.201101-0056OC.
6. Nhu NT, Heemskerk D, Thu do DA, et al. Evaluation of GeneXpert MTB/RIF for diagnosis of tuberculous meningitis. *J Clin Microbiol* 2014;52(1):226–33. doi:10.1128/JCM.01834-13.
7. Detjen AK, DiNardo AR, Leyden J, et al. Xpert MTB/RIF assay for the diagnosis of pulmonary tuberculosis in children: A systematic review and meta-analysis. *Lancet Respir Med* 2015;3(6):451–61. doi:10.1016/S2213-2600(15)00095-8.

8. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010;363(11):1005-15. doi:10.1056/NEJMoa0907847.
9. Lawn SD, Nicol MP. Xpert® MTB/RIF assay: Development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. *Future Microbiol* 2011;6(9):1067-82. doi:10.2217/fmb.11.84.
10. Horne DJ, Kohli M, Zifodya JS, et al. Xpert MTB/RIF and Xpert MTB/RIF Ultra for pulmonary tuberculosis and rifampicin resistance in adults. *Cochrane Database Syst Rev* 2019;6(6):CD009593. doi:10.1002/14651858.CD009593.pub4.
11. Division of Tuberculosis, Department of Disease Control. National Tuberculosis Control Program Guidelines, Thailand 2021. Bangkok: Aksorn Graphic and Design; 2021. (in Thai)
12. Horne DJ, Zifodya JS, Shapiro AE, et al. Xpert MTB/RIF Ultra assay for pulmonary tuberculosis and rifampicin resistance in adults and adolescents. *Cochrane Database Syst Rev* 2025;7(7):CD009593. doi:10.1002/14651858.CD009593.pub6.
13. Dorman SE, Schumacher SG, Alland D, et al. Xpert MTB/RIF Ultra for detection of mycobacterium tuberculosis and rifampicin resistance: A prospective multicentre diagnostic accuracy study. *Lancet Infect Dis* 2018;18(1):76-84. doi:10.1016/S1473-3099(17)30691-6.
14. Rangsiphanurat W, et al. Prevalence of tuberculosis and rifampicin-resistant tuberculosis using Xpert MTB/RIF Ultra in Samut Prakan Hospital from 2018 to 2022. *Journal of Huachiew Chalermprakiet University* [Internet] 2023 [cited 2026 Jan 5];27(1):88-98. Available from: <https://has.hcu.ac.th/xmlui/handle/123456789/6135>.
15. Gaikwad PC, Trivedi P, Pandey DK, et al. Analysis of rifampicin resistance of Mycobacterium tuberculosis detected by Xpert MTB/RIF assay in a tertiary health care hospital. *Int J Acad Med Pharm* 2024;6(2):582-6. doi:10.47009/jamp.2024.6.2.123.
16. Modongo C, Barilar I, Wang Q, et al. Tuberculosis variant with rifampin resistance undetectable by Xpert MTB/RIF, Botswana. *Emerg Infect Dis* 2023;29(11):2403-6. doi:10.3201/eid2911.230987.
17. Cox H, Dickson-Hall L, Ndjeka N, et al. Delays and loss to follow-up before treatment of drug-resistant tuberculosis following implementation of Xpert MTB/RIF in South Africa: A retrospective cohort study. *PLoS Med* 2017;14(2):e1002238. doi:10.1371/journal.pmed.1002238.
18. Albert H, Nathavitharana RR, Isaacs C, et al. Development, roll-out and impact of Xpert MTB/RIF for tuberculosis: What lessons have we learnt and how can we do better? *Eur Respir J* 2016;48(2):516-25. doi:10.1183/13993003.00543-2016.



JOURNAL OF **MEDICINE AND HEALTH SCIENCES**

VOLUME 33 • NUMBER 2 • AUGUST 2026

Published by

Faculty of Medicine, Srinakharinwirot University

📍 62 Village No.7, Rangsit-Nakhon Nayok Road,
Ongkharak Sub-district, Ongkharak District,
Nakhon Nayok 26120, Thailand

🌐 <https://med.swu.ac.th/research/>