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ทำแบบประเมิน
คลิกที่ปุ่มเพื่อทำแบบประเมิน

โครงการ
frail walk
4 weeks

สื่อความรู้
การดูแลสุขภาพ

รู้จักภาวะ
POCD กันเถอะ

วิธีใช้งาน

เมนู ▾

	1	2	3	4	5	6	7	8	9	10	11	12	
A	128	64	32	16	8	4	2	1	0.5	0.25	0.125	GC	} <i>Acinetobacter baumannii</i>
B	128	64	32	16	8	4	2	1	0.5	0.25	0.125	SC	
C	128	64	32	16	8	4	2	1	0.5	0.25	0.125	GC	
D	128	64	32	16	8	4	2	1	0.5	0.25	0.125	SC	} <i>Klebsiella pneumoniae</i>
E	128	64	32	16	8	4	2	1	0.5	0.25	0.125	GC	
F	128	64	32	16	8	4	2	1	0.5	0.25	0.125	SC	} <i>Pseudomonas aeruginosa</i>
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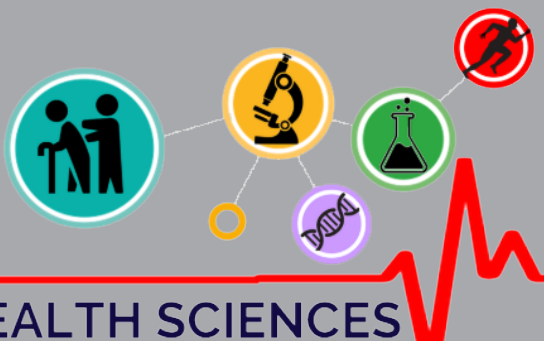
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Contents

Feasibility of a technology readiness level 6 Web application for monitoring postoperative cognitive dysfunction in older adults: a pilot study	1
<i>Chayanit Techareerat, Thanwai Pisalayon, Chanisara Thaomueangjai, Arunotai Siriussawakul, Nipaporn Sangarunakul, Chalita Jiraphorncharas, Phongchai Petsanghan, Patumporn Suraarunsumrit</i>	
Frozen storage stability of pre-diluted colistin microdilution plates for routine susceptibility testing	18
<i>Aschana Tirapattanun, Pintip Suchartlikitwong, Sunisa Chirakul</i>	
Immediate effects of kinesiology tape on pain and quadriceps muscle strength in individuals with patellofemoral pain syndrome	26
<i>Nipaporn Rigo, Atcharaphan Changkeaw</i>	
Effects of aerobic and combined exercise interventions on CD4 cell counts and VO₂max in people living with HIV: a systematic review and meta-analysis	35
<i>Sarayoot Mongkol, Chuenpak Saleesing, Ettipad Jaiyen, Tichaporn Sookkue, Sukanya Kreeinthong</i>	

Feasibility of a technology readiness level 6 web application for monitoring postoperative cognitive dysfunction in older adults: a pilot study

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KEYWORDS

Digital health;
Geriatric patients;
Long-term follow-up;
Postoperative
cognitive impairment;
Technology readiness
level.

ABSTRACT

Postoperative cognitive dysfunction (POCD) is a common yet under-recognized issue in older surgical patients. Long-term cognitive follow-up is often absent in standard care. The COVID-19 pandemic highlighted the need for remote monitoring tailored to aging populations. Therefore, this study presented a web-based platform, developed under World Health Organization's Integrated Care for Older People (ICOPE) framework and evaluated at Technology Readiness Level 6 (TRL-6). The objective of this study was to design and commission the development of the web application, and to assess its feasibility among older surgical patients within a clinical setting. The study was conducted in two phases: system development based on literature-guided risk factors and ICOPE principles, and a prospective cohort study to evaluate feasibility by assessing data completeness, user input capability, and follow-up adherence after surgery. Patients aged ≥ 60 years who underwent elective surgery under anesthesia were enrolled. Exclusion criteria were included having severe sensory deficits or inability to use a smartphone. POCD was defined as a ≥ 2 -point decline in the Montreal Cognitive Assessment (MoCA) score from baseline. This study found the platform featured (1) communication via a LINE official account (LINE OA), (2) risk screening with tailored educational video clips, and (3) automated reminders for postoperative assessments. Ten participants (mean age 70.90 ± 6.14 years; 70% female) were recruited between April and July 2024. Retention rates were 100% at 1 week and 70% at 3 months. POCD was observed in 2 of 10 participants at 1 week and 2 of 7 of participants at 3 months postoperatively. In conclusions, this TRL-6 pilot shows the feasibility of integrating a digital cognitive tool into perioperative care, with potential to enhance follow-up and early POCD detection in older patients.

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Introduction

Thailand is undergoing a demographic shift toward an aging population, leading to increased healthcare utilization among older adults. Age-related declines in immunity and physical resilience make this group more vulnerable to illness and surgical interventions compared to younger individuals⁽¹⁾. Cognitive decline, including mild cognitive impairment (MCI) and dementia, further increases healthcare demands and significantly affects both patients and their families⁽²⁾. In this context, cognitive vulnerability represents an important factor influencing postoperative outcomes in the aging population.

Postoperative complications, particularly postoperative cognitive dysfunction (POCD), are major concerns among older surgical patients, with reported incidence rates ranging from 9% to 46%, depending on diagnostic criteria, including timing of cognitive assessment, type of surgery, patient population, and diagnostic criteria^(3, 4). POCD primarily affects individuals aged 60 and older⁽⁵⁾, with approximately 40% showing signs within the first postoperative week⁽⁶⁾. About 10% continue to experience cognitive impairment at 3 months⁽⁷⁾, and some cases persist for up to 1 year⁽⁸⁾. POCD is associated with prolonged hospitalization, increased mortality, and lasting cognitive decline⁽⁹⁾.

Currently, Thailand lacks a standardized approach to the management and prevention of POCD. Early detection and risk management are critical and require coordinated care involving surgeons, geriatricians, anesthesiologists, and psychiatrists⁽¹⁰⁾. The absence of multidisciplinary coordination has led to fragmented care, potentially compromising patient safety and increasing healthcare costs. To address this gap, the Siriraj Integrated Perioperative Geriatric Center (SiPG) launched the country's first dedicated POCD clinic—an unprecedented initiative to provide structured pre- and postoperative cognitive care for older adults. Since 2018, SiPG has implemented a protocol-driven cohort study involving patients

aged 60 and above undergoing major surgery, with ongoing follow-up to assess POCD prevalence, quality of life, and readmission rates. However, POCD remains poorly recognized and often presents with subtle or nonspecific symptoms. Patients, families, and even healthcare professionals are generally unfamiliar with the condition. It is rarely diagnosed and lacks specific treatment protocols. In addition, its long-term consequences are not clearly understood. As a result, many patients enrolled in the research project failed to attend scheduled follow-up appointments at the POCD clinic.

The COVID-19 pandemic has profoundly disrupted healthcare delivery, leading to a reduction in in-person hospital visits and the postponement of elective surgeries, which in turn worsened outcomes among medically vulnerable populations⁽¹¹⁾. In response to these challenges, healthcare institutions increasingly adopted telemedicine to maintain continuity of care through remote consultations and follow-up services^(12,13). Despite this progress, technological barriers—particularly among older adults and their caregivers—have limited the reach and effectiveness of such digital solutions⁽¹⁴⁾.

To address these limitations, the SiPG identified the need for a scalable digital health approach that aligns with the self-care capacities of older adults and can be embedded into routine perioperative workflows. In line with the World Health Organization's Integrated Care for Older People (ICOPE) framework—particularly its focus on maintaining cognitive function and enabling community-based monitoring—a remote cognitive surveillance concept was proposed⁽¹⁵⁾. This approach aims to support early detection of POCD, empower patient engagement through structured self-reporting, and facilitate timely communication with healthcare professionals. Recent studies highlight the feasibility of using digital platforms—such as mobile apps, SMS-based systems, and interactive patient portals—to monitor cognitive trajectories, deliver tailored health education,

and maintain follow-up in aging populations, especially in resource-limited or post-discharge settings^(16, 17). Therefore, the objectives of this study were to (1) design and commission the development of the web application and (2) assess its feasibility and usability among older surgical patients within a clinical setting. The study hypothesized that the web application would enhance consultation and follow-up services and be feasible to implement.

Materials and methods

Study design

The research was conducted in 2 phases. Phase I involved the design and collaborative development of the web-based application. Phase II evaluated the system's feasibility in a clinical setting by assessing data completeness, user input capability (by older adults or caregivers), and follow-up adherence after major surgery.

Phase I

Phase I focused on the design and development of POCD clinic online, a web-based application grounded in the ICOPE framework proposed by the World Health Organization. This framework emphasizes person-centered care, self-management, and continuity across care transitions. Guided by literature on POCD risk factors and ICOPE principles, the research team specified the clinical and functional requirements of the system, which was then developed by a contracted health-tech company. The system's core features included self-reported risk assessment, automated follow-up reminders, and real-time communication capabilities. This development phase corresponded to Technology Readiness Level or TRL-5, reflecting validation in a simulated environment without real patient use.

Collaborative System Development

The development of the web-based application for POCD monitoring was conducted through a collaborative process between the research team and a contracted software

development company. The research team led the design by specifying clinical requirements and defining technical functionalities in a formal Terms of Reference document. The system was built around two core components. The first feature focused on patient- or caregiver-reported risk factors for POCD, including age, type of surgery, frailty status, and comorbidities such as hypertension, diabetes, and cardiovascular disease. These variables were selected based on clinical relevance and evidence from prior studies indicating their association with increased POCD risk^(18,19). An algorithm was developed to generate an individualized risk estimate and present the result in a user-friendly format to facilitate preoperative awareness and communication. The second feature comprised a clinician-facing dashboard that enabled real-time tracking of patient-reported cognitive assessments at scheduled intervals: preoperatively, 1 week postoperatively, and 3 months postoperatively. The platform also included automated prompts and reminders to support adherence to follow-up timelines. This integration of self-report data and clinical visualization aimed to improve continuity of care, support early detection of cognitive decline, and reduce loss to follow-up. The completed application was deployed and evaluated under real-world conditions, corresponding to TRL- 6, where a fully functional prototype was tested in a relevant clinical setting prior to broader implementation.

Phase II

Phase II involved a prospective cohort study at Siriraj Hospital. After internal testing, the platform was deployed in a clinical setting using a hybrid service model that combined digital tools with existing in-person workflows. A multi-disciplinary administrative team was established to manage the clinical aspects of the platform (Table 1). This team included research nurses, a clinical psychologist, a physiotherapist, and a health educator. Anesthesiologists and geriatricians were available for consultation when

clinical concerns arose. Research nurses were responsible for reviewing the completeness and accuracy of patient-submitted data, while the clinical psychologist verified and interpreted MoCA scores. On the technical side, the development team comprised software engineers and a dedicated support unit tasked with monitoring platform performance and resolving system

issues. A service-level agreement was established to ensure all technical problems were addressed within 24 hours. This restructured patient journey enabled remote cognitive follow-up and reduced the need for physical hospital visits. The implementation phase corresponded to TRL-6, indicating prototype testing in a real-world operational setting with actual patients and healthcare teams.

Table 1 Roles and responsibilities of the clinical and technical support teams

Team	Roles and responsibility
Research Nurses	- Review completeness and accuracy of submitted data - Monitor participant adherence and follow-up status
Clinical Psychologist	- Verify and interpret MoCA results - Provide mental health input when needed
Physiotherapist	- Contribute to functional assessment interpretation and patient guidance
Health Educator	- Support patient understanding of POCD and promote self-care practices
Anesthesiologists	- Provide clinical consultation related to anesthesia and perioperative cognitive risks
Geriatricians	- Provide comprehensive geriatric assessment and coordinate interdisciplinary responses
Software Development Team	- Design and maintain the platform - Implement risk algorithm and user interface components
Technical Support Unit	- Troubleshoot system issues - Ensure functionality and resolve problems within 24 hours

Data Collection and Assessments

The web-based system was developed to support structured data collection from 2 main sources: (1) self-reported input by patients or caregivers, and (2) clinically verified data entered by healthcare personnel. The data architecture and input interfaces were designed by the software development team under guidance from the research team to ensure usability and clinical relevance.

Self-Report Questionnaires

Participants or their caregivers completed a series of standardized questionnaires at 3 timepoints: preoperatively, 1 week postoperatively, and 3 months postoperatively. These included

a demographic questionnaire, the Montreal Cognitive Assessment (MoCA), the Modified Informant Questionnaire on Cognitive Decline in the Elderly (Modified IQCODE), Basic Activities of Daily Living (BADL), and Instrumental Activities of Daily Living (IADL) and European Quality of Life 5 Dimensions 5 Levels (EQ-5D-5L). POCD was defined as a decline of 2 or more points from the baseline MoCA score, in line with the standard deviation (SD = 2.14) reported in the Thai MoCA validation study⁽²⁰⁾ and supported by previous literature⁽⁹⁾. This definition aligns with the 2018 international consensus on perioperative neurocognitive disorders, which classifies POCD within the DSM-5 framework as part of a continuum encompassing

delayed neurocognitive recovery (within 30 days postoperatively) and postoperative neurocognitive disorder (from 30 days to 12 months following anesthesia and surgery)⁽¹⁸⁾. Independent BADL is indicated by a score above 70, while independent IADL is defined as scores greater than 5 for males and 8 for females⁽²¹⁾. High quality of life is indicated by an EQ-5D-5L value above 0.85⁽²²⁾.

Clinically Verified Data Input

Upon hospital admission, healthcare personnel were responsible for conducting and entering postoperative delirium assessments into the system. Depending on the clinical condition of each patient, research nurses conducted delirium screening between postoperative days 1 to 3 using either the 4 A's Test (4AT) at general wards or the Confusion Assessment Method for the Intensive Care Unit (CAM-ICU) at the ICU. These assessments ensured the accuracy and completeness of clinical data that could not be self-reported.

Ethical approval

This study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (COA no. [Si 750/2022]).

Participants

This pilot study's sample size was determined based on recommendations from previous methodological studies⁽²³⁾. Based on this guidance, a total of 10 participants were considered adequate for evaluating the feasibility and preliminary effectiveness of the web-based application.

Participants in the study were patients aged over 60 years who were scheduled for moderate to major elective surgery under regional or general anesthesia at Siriraj Hospital from April to July 2024. Eligibility required that either the patient or a caregiver had access to, and basic proficiency in, using online communication platforms such as LINE video call, Zoom, or Webex. Patients were excluded if they had neurological conditions (e.g., brain tumor, prior cerebrovascular disease, paralysis), were bedridden, had severe visual or hearing impairments that had not been previously

corrected or treated (e.g., absence of eyeglasses or hearing aids), were unable to communicate in Thai, or lacked access to online follow-up services. Informed consent was obtained from all participants.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics version 22 (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to summarize demographic characteristics, and the incidence of POCD was reported as frequencies and percentages.

Results

This study reported on the development, deployment, and preliminary implementation of a web-based application named POCD clinic online designed to support cognitive monitoring and perioperative care in older adults undergoing surgery. Developed according to the study's primary objective, POCD clinic online incorporated 3 primary features aimed at improving communication, risk identification, and follow-up adherence.

Feature 1: Secure and Accessible Communication Channel

The system facilitated secure communication between research staff and patients or caregivers via the LINE official account (LINE OA), providing timely guidance and support (Figure 1A). LINE was selected as the primary communication channel due to its widespread use in Thailand; it is the most used messaging application in the country, with particularly high adoption rates among older adults who own smartphones. Studies have shown that Thai older adults are generally familiar with LINE and find it easy to use for health-related communication, including messaging, video calls, and receiving reminders⁽²⁴⁾. Leveraging an existing, user-friendly platform enhanced accessibility and reduced barriers to digital engagement. Access to the application required identity verification through a one-time password to ensure data security and confidentiality (Figure 1B).

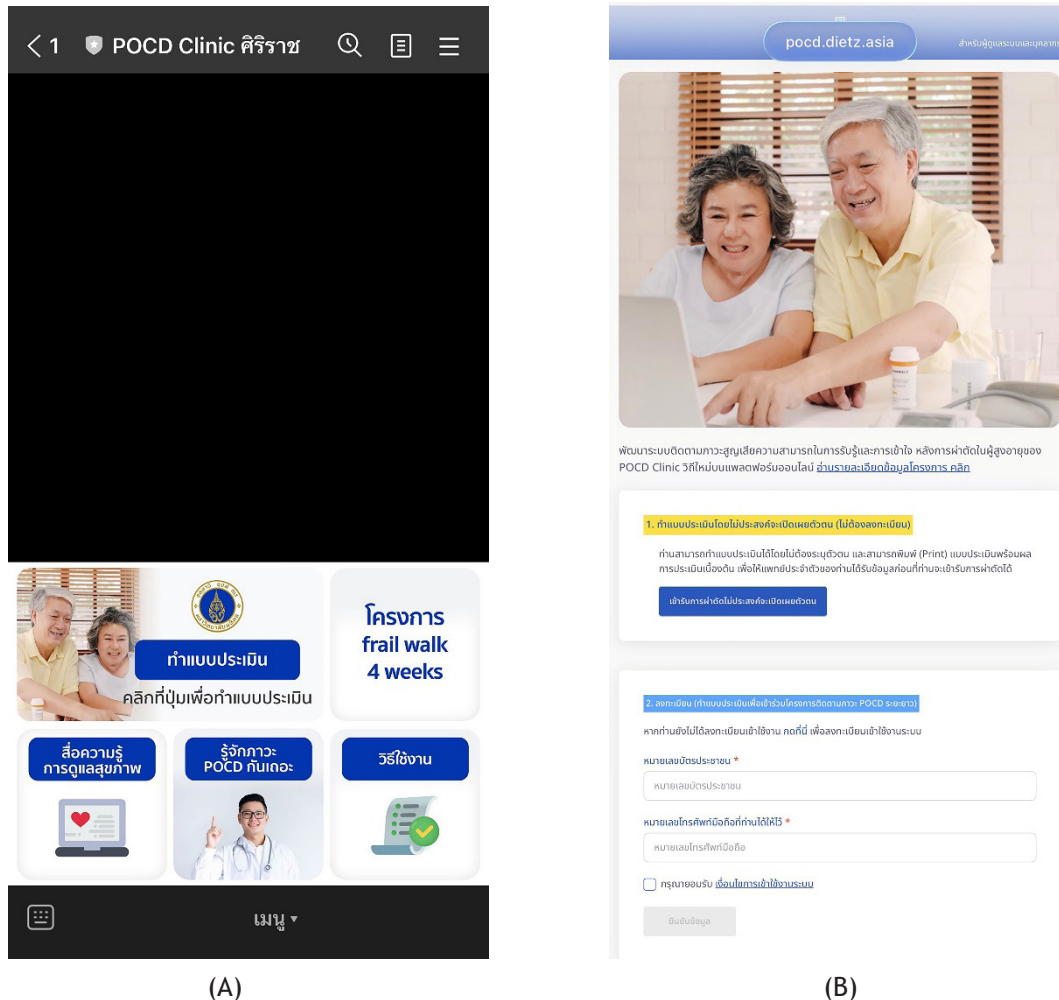


Figure 1 Screenshot of postoperative cognitive dysfunction online clinic
 (A) POCD clinic Siriraj LINE official account
 (B) POCD clinic online web application page

Abbreviation: POCD, post-operative cognitive dysfunction

Feature 2: Risk Stratification and Cognitive Screening Module

The platform included a risk screening module that allowed users to self-report POCD-related risk factors. The algorithm was developed based on variables identified through a review of relevant literature. These included frailty—assessed using the Simple FRAIL questionnaire completed by patients or caregivers—and major comorbidities such as cardiovascular disease^(25,26). Participants received different assessment links which contain all self-report at each follow-up

time point. Once submitted, the data were automatically processed to generate an individualized risk profile without requiring manual calculation.

Participants identified as high-risk were immediately provided with targeted educational video clips. These videos, developed by specialists from the Faculty of Medicine Siriraj Hospital, aimed to improve understanding of POCD, promote appropriate preventive behaviors, and encourage early recognition and timely management. Both patients and caregivers were notified of the risk

status and guided through self-directed education to enhance preparedness and engagement in the perioperative period.

In addition to risk screening, the system incorporated cognitive function assessments using MoCA – a globally validated tool widely used for detecting mild cognitive impairment. The platform

allowed patients to complete the assessment independently online. Test results were then verified by trained professionals, including physicians, research nurses, or clinical psychologists with experience in administering and interpreting MoCA (Figure 2).

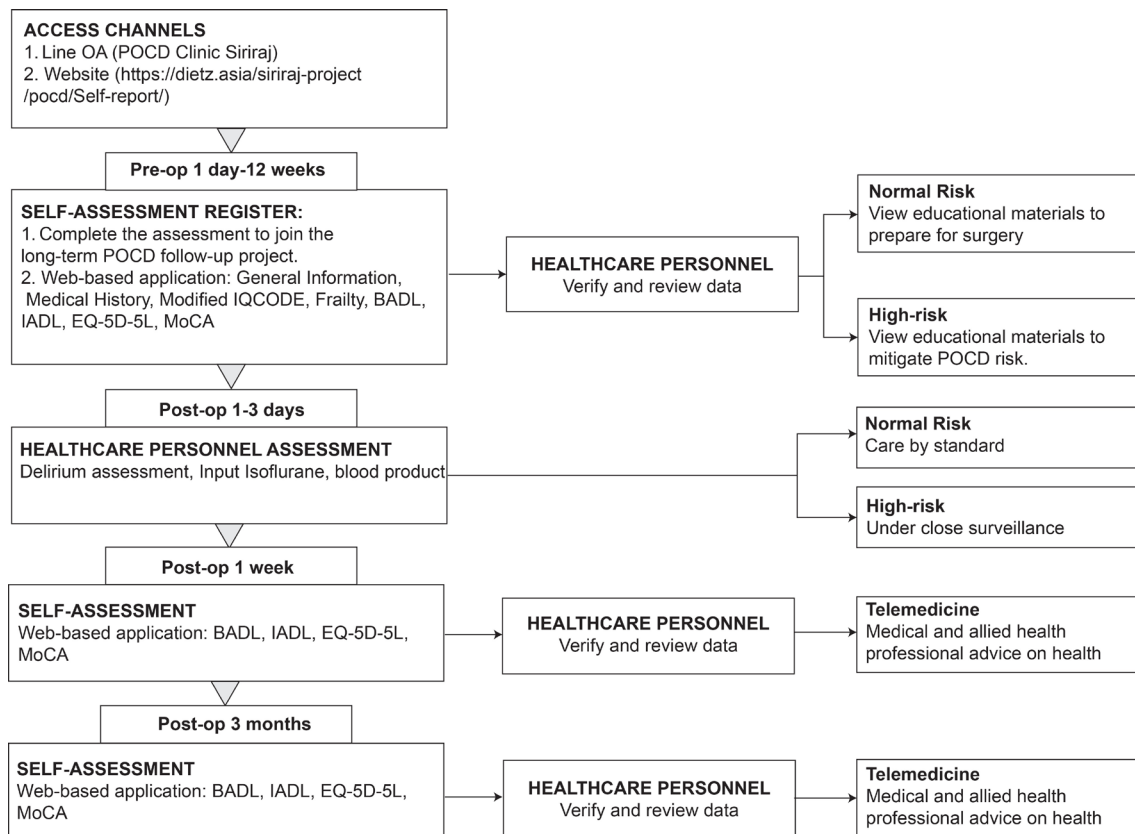


Figure 2 Patients' journey in postoperative cognitive dysfunction clinic web application

Abbreviations: POCD, post-operative cognitive dysfunction; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; BADL, Basic Activities of Daily Living; IADL, Instrumental Activities of Daily Living; EQ-5D-5L, European Quality of Life 5 Dimensions 5 Levels; MoCA, Montreal Cognitive Assessment

Feature 3: Automated Long-Term Cognitive Follow-Up

This feature addresses a critical gap in conventional perioperative care—namely, the lack of structured long-term follow-up for POCD. As highlighted in the introduction, POCD is often

under-monitored due to logistical challenges, particularly for older patients who face difficulties attending in-person hospital visits. POCD clinic online was designed as a digital solution to facilitate continuous monitoring through remote, self-administered assessments. The system

incorporated automated reminders prompting participants to complete cognitive and functional evaluations at key postoperative milestones. Participants completed assessments at 3 defined timepoints: preoperatively, and at 1 week, and 3 months postoperatively. Specifically, MoCA assessments were scheduled at 1 week and 3 months, while IADL was assessed at 1 week and 3 months. To support timely engagement, the platform issued notifications to patients or caregivers when assessments were due. This mechanism ensured data completeness and improved adherence to follow-up protocols.

In parallel, data submitted through the platform were reviewed and verified by healthcare personnel to ensure clinical accuracy. The clinical team had real-time access to a dashboard interface displaying patient data. When a participant's results indicated high POCD risk, the system flagged the case for close monitoring. This enabled timely outreach and proactive intervention, thereby supporting individualized recovery and reducing the likelihood of missed diagnoses. Once the data had been verified, participants were contacted via LINE message or video call to receive individualized guidance from a health professional. The full digital follow-up process is illustrated in figure 2.

Participant Engagement and Follow-Up Outcomes

Phase II involved feasibility testing of the POCD clinic online web application with

real patients in a clinical setting. Demographic characteristics are summarized in Tables 2 and 3. Participants were aged 61-82 years. Three male participants were included in this pilot study. Half of the participants had a normal body mass index. The most common comorbidities were dyslipidemia and cancer. Three participants presented with a risk of POCD. Frailty and pre-frailty were observed among seven participants. All participants had independent basic activities of daily living, while three participants had limitations in higher activities of daily living. Conversely, half of the participants exhibited a high level of health-related quality of life.

The completeness of the data is shown in Table 4. A total of 10 patients scheduled for elective moderate-to-major surgery were screened for eligibility. All 10 participants were recruited and successfully accessed and participated in POCD clinic online during the preoperative period. At the 1-week postoperative timepoint, all participants remained engaged with the system and completed the scheduled assessments. At the 3-month postoperative timepoint, 7 out of 10 participants continued using the platform and submitted all follow-up data. Three participants did not complete the self-assessment. Of these, 2 participants finished IADL, EQ-5D-5L, and BADL, while 1 did not complete any self-assessment tasks in the system.

Table 2 Baseline characteristics of study participants (n=10)

Variables	Values
Sex (female): n(%)	7(70)
Age: years ^a	70.9 ± 6.14
Education level over 6 years: n(%)	6(60)
Body mass index: kg/m ^{2a}	24.14 ± 3.83
Underweight: n(%)	1(10)
Normal: n(%)	5(50)
Overweight: n(%)	4(40)
Comorbidities	
Cancer: n(%)	4(40)
Dyslipidemia: n(%)	4(40)
Cardiovascular disease: n(%)	1(10)
Hypertension: n(%)	3(30)
Diabetes mellitus: n(%)	3(30)
Warfarin use: n(%)	2(20)
Liver disease: n(%)	1(10)
Asthma: n(%)	1(10)
Visual impairment: n(%)	1(10)
Surgery type	
Intrathoracic: n(%)	6(60)
Orthopedic: n(%)	2(20)
Abdominal: n(%)	1(10)
Breast: n(%)	1(10)
Cognitive and functional assessments	
Modified IQCODE above 3.44: n(%)	2(20)
Frailty	
Not frail: n(%)	3(30)
Pre-frail: n(%)	5(50)
Frail: n(%)	2(20)
EQ-5D-5L score above 0.85: n(%)	5(50)
BADL above 70: n(%)	10(10)
IADL male below 5 and female below 8: n(%)	3(30)

Note: ^a indicate the data are presented by mean ± SD

Abbreviations: IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; EQ-5D-5L, European Quality of Life 5 Dimensions 5 Levels; BADL, Basic Activities of Daily Living; IADL, Instrumental Activities of Daily Living

Table 3 Individual characteristics of participants

Case	Sex (M/F)	Age (years)	Body mass index	Education level	Comorbidities	Surgery type	POCD risk	Modified IQCODE	Frailty	EQ-5D-5L	BADL	IADL
1	M	66	Over	Less than 6 years	HT, CVS, DLP, warfarin	Thoracic	At risk	No risk	Normal	Full-good health	Independent	Independent
2	F	67	Over	Less than 6 years	HT	Thoracic	Low risk	No risk	Pre-frail	Reduced quality of life	Independent	Dependent
3	F	74	Normal	Less than 6 years	DM, DLP, CA	Breast	Low risk	No risk	Pre-frail	Full-good health	Independent	Independent
4	M	75	Over	More than 6 years	CA, visual impairment	Orthopedic	Low risk	No risk	Pre-frail	Reduced quality of life	Independent	Independent
5	F	72	Normal	More than 6 years	HT, CA, warfarin	Thoracic	At risk	No risk	Normal	Full-good health	Independent	Independent
6	F	66	Over	More than 6 years	DM, DLP, Liver, CA	Abdominal	Low risk	No risk	Normal	Full-good health	Independent	Independent
7	F	70	Normal	Less than 6 years	DM, DLP, asthma	Thoracic	At risk	At risk	Frail	Reduced quality of life	Independent	Dependent
8	M	76	Normal	More than 6 years	Nil	Thoracic	Low risk	No risk	Frail	Full-good health	Independent	Independent
9	F	61	Normal	More than 6 years	Nil	Thoracic	Low risk	No risk	Pre-frail	Reduced quality of life	Independent	Independent
10	F	82	Under	More than 6 years	Nil	Orthopedic	Low risk	At risk	Pre-frail	Reduced quality of life	Independent	Dependent

Note: Nil indicates the participant absence of underlying diseases.

Abbreviations: POCD, post-operative cognitive dysfunction; IQCODE, Informant Questionnaire on Cognitive Decline in the Elderly; EQ-5D-5L, European Quality of Life 5 Dimensions 5 Levels; BADL, Basic Activities of Daily Living; IADL, Instrumental Activities of Daily Living; HT, hypertension; CVS, cardiovascular disease; DLP, dyslipidemia; Warfarin, Warfarin use; DM, diabetes mellitus; CA, carcinoma; Liver, liver disease

Table 4 Data completeness by the participants

Case	Preoperative					One-week follow-up					Three-month follow-up					Completeness (%)
	MoCA	BADL	IADL	EQ-5D-5L	MoCA	BADL	IADL	EQ-5D-5L	MoCA	BADL	IADL	EQ-5D-5L				
1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
2	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	91.67		
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
6	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
7	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
8	✓	✓	✓	✓	✓	✓	✓	✓	×	×	×	×	×	66.67		
9	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	100		
10	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓	✓	91.67		
Completeness (%)	100	100	100	100	100	100	100	100	70	90	90	90	90			

Note: ✓ indicates that participants had complete data, x indicates that participants had not complete data

Abbreviations: MoCA, Montreal Cognitive Assessment; BADL, Basic Activities of Daily Living; EQ-5D-5L, European Quality of Life 5 Dimensions 5 Levels; IADL, Instrumental Activities of Daily Living

Incidence of Postoperative Delirium and POCD

This section reported clinical outcomes from the feasibility study. Preoperative screening with the Modified IQCODE identified 2 of 10 participants at risk for underlying dementia. Delirium and cognitive outcomes over time are summarized in Table S1 (Supplementary Material). Postoperative delirium occurred in 2 participants undergoing thoracic surgery within the first 3 postoperative days. POCD was identified in 2 of 10 patients at 1 week and in 2 of 7 patients at the 3-month follow-up; one participant exhibited POCD at both time points.

Mean MoCA scores declined slightly from 21.9 preoperatively to 21.2 at 1 week and returned to baseline by 3 months, while POCD cases showed larger declines. Mean IADL scores decreased from 7.40 preoperatively to 6.50 at 1 week and recovered to 7.29 at 3 months, indicating a temporary functional decline. BADL scores followed a similar pattern, with most participants remaining independent; reduced independence was observed in one POCD case at 3 months. EQ-5D-5L utility scores declined from a mean of 0.78 at baseline to 0.71 at 1 week and subsequently improved to 0.79 at 3 months. All patients with POCD reported a reduced quality of life at 1 week. At follow-up, all participants received individualized feedback and recommendations.

Discussion

This study demonstrates that integrating digital platforms into perioperative care is both feasible and potentially effective. The 3 main features of POCD clinic online—secure communication via LINE OA, automated risk stratification with tailored education, and scheduled cognitive assessments—were well-received and led to substantial patient engagement. At 1 week and 3 months postoperatively, retention rates were 100% and 70%, respectively, which compares favorably to similar digital health interventions that often face attrition rates exceeding 40%⁽²⁷⁾. Therefore, the use of LINE OA, a platform already familiar

to most Thai older adults, likely contributed to the ease of access and adoption, consistent with findings from local studies on mobile health acceptance⁽²⁴⁾.

Furthermore, the platform successfully identified high-risk patients using preoperative self-reported data. This screening proved effective: 2 of the high-risk participants developed POCD at 1 week and 3 months, compared to only 1 participant from the low-risk group. This highlights the utility of early digital screening in prompting proactive responses from care teams and tailoring postoperative interventions. The ability to deliver educational content directly to patients and caregivers via video clips further supports informed engagement and encourages behaviors that may mitigate cognitive decline. This feature is consistent with findings from a previous study that developed a web-based application for osteoarthritis risk screening in Thai older adults, which reported high user satisfaction due to its ability to provide effective risk assessment and individualized advice⁽²⁸⁾.

The COVID-19 pandemic catalyzed the global shift toward digital health, highlighting its value in maintaining continuity care, particularly for vulnerable populations such as older adults⁽²⁹⁾. In parallel, the rise of aging societies has brought increased attention to cognitive and functional decline in postoperative patients, particularly those undergoing major surgery. POCD is now recognized as a common yet often overlooked condition that significantly affects quality of life and independence in older adults^(18,30). Traditionally, perioperative care has focused on physical recovery, with limited cognitive follow-up due to logistical barriers, workforce limitations, and lack of structured monitoring systems⁽¹⁰⁾.

In response, the ICOPE framework has emphasized a shift toward person-centered, community-supported, and technology-assisted care models⁽¹⁾. ICOPE advocates for empowering older individuals to participate actively in self-care and decision-making, supported by digital tools that promote early detection and sustained

monitoring of cognitive decline⁽¹⁾. Within this context, the POCD clinic online platform developed and tested in this study aligns with these principles by offering a scalable, web-based solution to long-standing gaps in postoperative cognitive monitoring. The platform integrates multiple assessment components administered at predefined perioperative time points, with each assessment requiring a limited and manageable amount of time. While the longitudinal follow-up period—including assessments up to 3 months postoperatively—may increase the risk of loss to follow-up, such extended monitoring is essential for older adults, who remain vulnerable to delayed cognitive changes after surgery. In addition, some participants who demonstrated normal cognitive scores at early postoperative assessments may have perceived limited personal benefit from later evaluations, potentially affecting retention. Nevertheless, the platform was intentionally designed to support continuous engagement of both patients and caregivers throughout critical preoperative and postoperative periods, thereby facilitating sustained monitoring and reinforcing the principles of person-centered and proactive geriatric care.

Despite these strengths, challenges remain. Loss to follow-up at 3 months suggests barriers to sustained digital engagement among older adults, potentially due to health deterioration, low digital literacy, or inadequate caregiver support. Previous literature has similarly reported that age-related sensory decline, cognitive impairment, and limited familiarity with mobile technologies can negatively impact digital health adherence in geriatric populations⁽¹⁵⁾. Strategies to address these challenges may include simplified user interfaces, caregiver-assisted reporting, and incentive-based engagement mechanisms.

Functional outcomes also merit further attention. The observed decline in IADL, BADL, and EQ-5D-5L utility scores at 1 week that recovered at 3 months suggests that POCD may contribute to broader declines in daily functioning and quality

of life, as the IADL, BADL, and EQ-5D-5L scores in POCD patients were lower than in non-POCD patients at 3 months. Given the association between cognitive and physical impairment in older adults, integrated rehabilitation approaches that address both domains may be warranted. These could include digital physical therapy modules, remote cognitive training, or coordinated follow-up with multidisciplinary teams.

This study has several limitations. The sample was drawn from a university hospital and included participants with baseline access to smartphones and high digital literacy, limiting generalizability. Self-reported data may be subject to recall or social desirability bias, particularly in cognitive assessments like MoCA. Moreover, the definition of POCD as a ≥ 2 -point decline in MoCA, while practical, may not capture all clinically relevant cognitive changes, and lacks universal standardization across specialties. Furthermore, this study is the lack of data identifying whether responses were submitted by patients or caregivers. Therefore, the relative contribution of each group could not be assessed. Future studies should collect this information to better inform program development and implementation.

Another important limitation is the lack of a control group or comparison arm, which prevents firm conclusions about the platform's effectiveness versus standard care. Future studies should consider randomized controlled designs to evaluate clinical outcomes more rigorously. Additionally, long-term cognitive trajectories beyond 3 months were not captured, although POCD has been documented to persist for up to 1 year or longer in some patients⁽³¹⁾. In future iterations, expansion of the platform's functionality to include other ICOPE domains—such as mobility, nutrition, and psychological well-being—could enhance its utility as a comprehensive aging-support tool⁽¹⁾. Integration with national health records and interoperability with hospital systems would further facilitate continuity of care and data-driven planning.

Conclusion

In summary, POCD clinic online represents a promising innovation for digital perioperative cognitive care in older adults. It addresses critical gaps in follow-up, enhances patient engagement, and aligns with global healthy aging strategies. While refinement is needed, particularly to improve long-term adherence and broader inclusivity, the platform lays a foundation for scalable, technology-supported models of geriatric care.

Take home messages

POCD Clinic Online is a WHO ICOPE-aligned digital platform evaluated at TRL-6. This pilot explored perioperative use in older adults, demonstrating feasible remote monitoring, high retention, and detection of postoperative cognitive change. Findings indicate potential value for technology-supported geriatric surgical care and justify larger-scale evaluation in future clinical studies.

Conflicts of interest

The authors declare no conflict of interest.

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Data availability

Data available on request from the authors.

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Supplementary material

Table S1 Delirium, POCD, cognitive, and functional assessments over time

Variable	Preoperative				One-week follow up (n=10)				Three-month follow up (n=7)				POD		POCD at	
	MoCA	BADL	IADL	EQ-5D-5L	MoCA	BADL	IADL	EQ-5D-5L	MoCA	BADL	IADL	EQ-5D-5L	EQ-5D-5L	2(20)	1 week	3 months
Total values ^a	21.90 ± 4.12	94.00 ± 9.66	7.40 ± 0.70	0.78 ± 0.28	21.20 ± 4.16	91.50 ± 8.52	6.50 ± 1.78	0.71 ± 0.28	21.71 ± 5.06	95.71 ± 11.34	7.29 ± 1.25	0.79 ± 0.39	0.79 ± 0.39	2(20)	2(20)	2(28.57)
No POCD ^a	-	-	-	-	21.88 ± 4.02	92.50 ± 9.26	6.38 ± 1.923	0.69 ± 0.31	24.20 ± 3.19	92.50 ± 9.26	7.40 ± 1.34	0.94 ± 0.09	0.94 ± 0.09			
POCD ^a	-	-	-	-	18.50 ± 4.95	87.50 ± 3.54	7.00 ± 1.41	0.80 ± 0.00	15.50 ± 2.12	87.50 ± 3.54	7.00 ± 1.41	0.42 ± 0.72	0.42 ± 0.72			
Individual value																
1	27	80	7	1.00	22	85	8	0.80	14	100	6	0.93	0.93	No	present	present
2	15	80	6	0.11	15	80	6	0.08	N/A	100	8	0.25	0.25	No	No	N/A
3	19	100	8	0.96	18	100	4	0.92	20	100	5	1.00	1.00	No	No	No
4	20	100	8	0.82	22	100	8	0.82	25	100	8	1.00	1.00	No	No	No
5	23	100	8	1.00	23	100	8	1.00	22	100	8	0.80	0.80	No	No	No
6	27	100	8	0.97	28	95	7	0.83	28	100	8	0.90	0.90	No	No	No
7	26	80	7	0.52	25	85	7	0.52	17	70	8	0.09	0.09	No	No	present
8	18	100	7	0.86	15	90	6	0.80	N/A	N/A	N/A	N/A	N/A	present	present	N/A
9	24	100	8	0.81	23	100	8	0.87	26	100	8	1.00	1.00	present	No	No
10	20	100	7	0.80	21	80	3	0.46	N/A	75	1	0.80	0.80	No	No	N/A

Note: ^a The data are presented by mean ± SD

Abbreviations: MoCA, Montreal Cognitive Assessment; BADL, Basic Activities of Daily Living; IADL, Instrumental Activities of Daily Living; EQ-5D-5L, European Quality of Life 5 Dimensions 5 Levels; POD, post-operative delirium; POCD, post-operative cognitive dysfunction; N/A, Not Applicable

Frozen storage stability of pre-diluted colistin microdilution plates for routine susceptibility testing

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KEYWORDS

Colistin;
Broth microdilution (BMD);
Antimicrobial susceptibility testing;
Frozen storage stability;
Minimum inhibitory concentration (MIC).

ABSTRACT

Broth microdilution (BMD) is the reference method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for determining colistin susceptibility testing; however, its routine implementation in clinical laboratories remains challenging due to factors such as storage conditions and technical variability. To provide a more practical and standardized approach, this study evaluated the stability of pre-diluted colistin stored at -20°C in a 96-well microdilution plate format. Serial twofold dilutions of colistin in Mueller-Hinton broth were prepared, frozen, and tested at 15-day intervals over 90 days using *Klebsiella pneumoniae* (colistin-resistant clinical isolate), *Acinetobacter baumannii* (colistin-intermediate isolate), and *Pseudomonas aeruginosa* ATCC27853 (quality control strain). Minimum inhibitory concentrations (MICs) were determined according to CLSI guidelines to monitor changes in antimicrobial activity during storage. The MIC values of all strains remained consistent throughout the 90-day period, indicating that colistin retained its biological activity under frozen storage. These findings demonstrate that colistin microdilution plates stored at -20 °C remain stable and reliable for at least three months, offering laboratories a convenient and reproducible method for preparing ready-to-use colistin BMD plates to support standardized susceptibility testing.

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Introduction

Colistin, also known as polymyxin E, is a member of the polymyxin class of antibiotics currently used in human medicine. It was first discovered in the 1940s and exhibits potent activity against gram-negative bacterial infections, particularly *Enterobacteriales*⁽¹⁾. Colistin is polycationic molecule containing both hydrophilic and lipophilic regions. These enable it to interact with the outer membrane of gram-negative bacteria by competitively displacing divalent cations, especially magnesium and calcium, from the membrane lipids. This disruption leads to increased membrane permeability, resulting in cell lysis and bacterial death. In addition, colistin can bind to and neutralize bacterial lipopolysaccharides, conferring anti-endotoxin properties⁽²⁾. Despite its effectiveness, the use of colistin declined for several decades due to reports of significant nephrotoxicity and neurotoxicity⁽²⁻⁴⁾ and the availability of safer and more potent antibiotics. However, the global emergence of multidrug-resistant (MDR) gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, has renewed clinical interest in colistin as a last-resort therapeutic agent⁽⁵⁻⁸⁾.

Accurate antimicrobial susceptibility testing (AST) is essential to ensure appropriate antibiotic therapy and to monitor the emergence of resistance⁽⁹⁾. Several AST methods are routinely used in clinical microbiology laboratories, including automated systems, disk diffusion, and gradient diffusion (E-test). However, both the Clinical and Laboratory Standards Institute (CLSI)⁽¹⁰⁾ and the European Committee on Antimicrobial Susceptibility Testing (EUCAST)⁽¹¹⁾ recommend broth microdilution (BMD) as the only reliable method for determining colistin susceptibility, as colistin's large cationic structure prevents accurate diffusion in agar-based assays⁽¹²⁾. Although BMD is the reference method, its use in routine clinical setting is limited by complexity of the procedure, which involves multiple

preparation steps. Factors such as inoculum preparation, culture medium, antibiotic concentration, and laboratory staff expertise can all influence the resulting minimum inhibitory concentration (MIC) values^(13,14). To make BMD more practical for routine use, laboratory could prepare pre-diluted colistin microdilution plates in advance and store them frozen. Therefore, determining the stability of colistin stored at -20 °C is critical to ensure the accuracy and reproducibility of MIC testing over time.

To our knowledge, while the stability of several reconstituted antibiotics under various storage conditions has been investigated using BMD method, similar data for colistin are lacking^(13,15,16). The present study aimed to evaluate the stability of colistin stored at -20 °C in 96-well microdilution plate format using the BMD method.

Materials and methods

Bacterial strains

Three bacterial strains were used in this study: *Pseudomonas aeruginosa* ATCC 27835 (quality control strain) and two clinical isolates, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, with known colistin MIC values, obtained from the Microbiology Laboratory, King Chulalongkorn Memorial Hospital. All strains were stored at -80 °C in the Department of Microbiology, Faculty of Medicine, Chulalongkorn University.

Preparation of Colistin and bacterial suspensions

Colistin sulfate salt (Glentham Life Sciences, United Kingdom) was prepared as a stock solution at a concentration of 1,024 µg/mL in sterile distilled water. Bacterial colonies from overnight growth on sheep blood agar (Biomedica, Thailand) were suspended in 0.85% (w/v) sodium chloride solution and adjusted to a 0.5 McFarland standard (OD₆₀₀ of 0.08 - 0.10), measuring using a Bio-Rad SmartSpec™ Plus spectrophotometer; Bio-Rad Laboratories, Hercules, CA, USA), corresponding to approximate 1.5 x 10⁸ CFU/mL. The 0.5 McFarland suspension was then diluted 1:100

in cation-adjusted Mueller-Hinton broth (CAMHB; BD DIFCO™, Le Pont de Claix, France) to obtain a working inoculum of approximately 1×10^6 CFU/mL.

Evaluation of the stability of freezing colistin by using time-varying BMD

The broth microdilution (BMD) assay was performed according to CLSI guidelines⁽¹⁷⁾. Briefly, the 1024 µg/mL colistin stock solution was first diluted 1:1 in CAMHB to obtain a 512 µg/mL working solution. Serial twofold dilutions were then prepared in untreated flat-bottom 96-well microdilution plates (Wuxi NEST Biotechnology, China) by dispensing 50 µL of colistin solution in well containing 50 µL of CAMHB, resulting in colistin concentrations ranging from 0.25 to 256 µg/mL.

Following preparation of the antibiotic dilutions, 50 µL of bacterial working inoculum was added to each well, yielding final colistin concentrations ranging from 0.125 to 128 µg/mL and a final inoculum of approximately 5×10^5 CFU/mL, in accordance with CLSI recommendations.

Preparation of pre-diluted BMD trays required approximately 20-30 minutes per 96-well plate, including drug dilution and dispensing. The prepared microdilution plates were sealed, labeled, and stored at -20 °C until use. At 15-day intervals over a 90-day period, the stored plates were thawed once at room temperature (22 - 25 °C) for approximately 20 minutes and used immediately. Plates were not subjected to repeated freeze-thaw cycles, and stability was evaluated exclusively under these defined storage and handling conditions.

Each assay included a growth control well (bacteria without antibiotic; GC) and a sterility control well (broth only; SC) Figure 1. After inoculation, plates were incubated at 35 ± 2 °C for 18 - 24 hours. The MIC was defined as the lowest concentration of colistin that completely inhibited visible bacterial growth. A change in MIC by greater than ± 1 twofold dilution from the initial value was interpreted as a loss of biological activity. All experiments were performed in duplicate (technical replicates) and repeated independently three times (biological replicates).

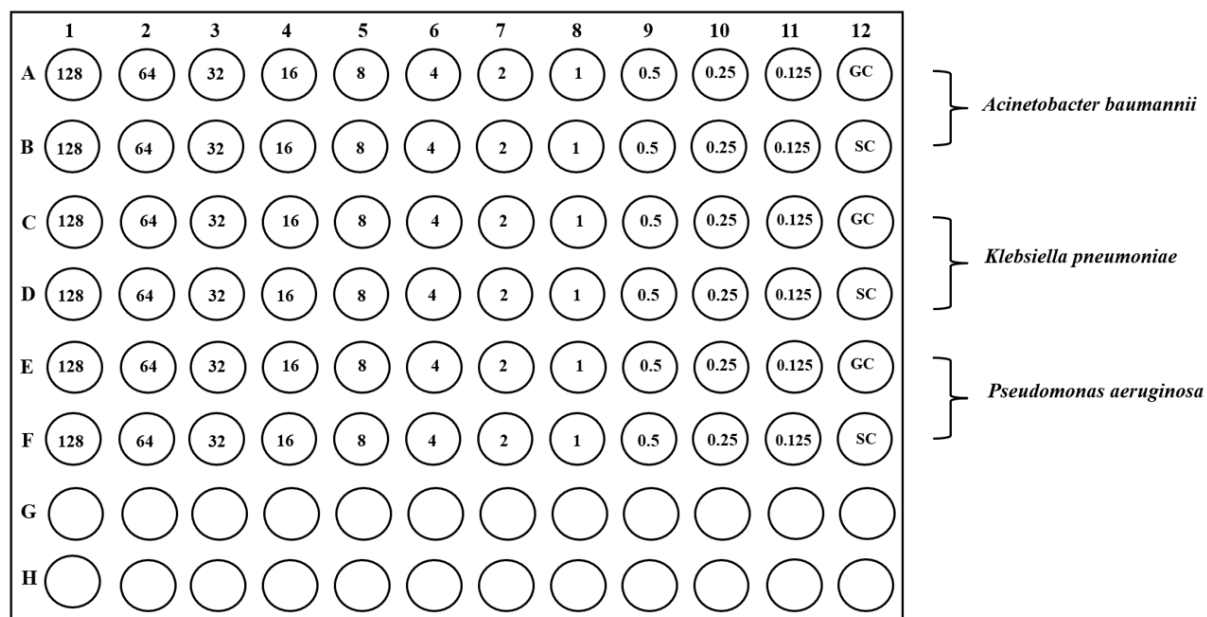


Figure 1 Layout of the 96-well broth microdilution (BMD) plate used for evaluation of colistin stability during frozen storage

The schematic illustrates the standardized plate configuration used throughout the study. Numbers indicate the final colistin concentrations ($\mu\text{g/mL}$) after addition of the bacterial inoculum, prepared as serial two-fold dilutions in cation-adjusted Mueller-Hinton broth (CAMHB), ranging from 0.125 to 128 $\mu\text{g/mL}$. Each bacterial strain was tested in two adjacent rows, with growth control wells (inoculated broth without colistin; GC) and sterility control wells (broth only; SC) included on each plate. Plates were sealed with adhesive film, stored at -20°C , and thawed at predefined time points for MIC determination.

Quality control

Quality control of inoculum density was performed in accordance with CLSI M07⁽¹⁷⁾. Briefly, 10 μL of the 0.5 McFarland suspension was transferred into 10 mL of sterile normal saline, yielding an estimated concentration of approximately 1×10^5 CFU/mL. Subsequently, 100 μL of this suspension was transferred into 10 mL of sterile normal saline, and 100 μL of the final dilution was spread on Mueller-Hinton agar (MHA; BD DIFCO™, Le Pont de Claix, France). Plates were incubated at $35 \pm 2^\circ\text{C}$ for 18 - 24 hours and colonies were enumerated. An acceptable colony count was approximately 150 colonies per plate. All assays were performed in triplicate.

Ethics statement

This study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Chulalongkorn University (IRB No. 0737/67, COE No. 063/2024).

Results

The stability of frozen, pre-diluted colistin microdilution plates was evaluated over a 90-day period using the broth microdilution (BMD) method described previously. Two clinical isolates with known colistin MIC values, *Klebsiella pneumoniae* (colistin-resistant, MIC = 64 $\mu\text{g/mL}$) and *Acinetobacter baumannii* (colistin-intermediate, MIC = 1 $\mu\text{g/mL}$), were tested along with *Pseudomonas aeruginosa* ATCC27853 as the quality control strain. Experiments were performed in technical duplicate wells and independently repeated three times on separate occasions (biological replicates). Individual MIC values from all biological replicates are shown in table 1.

Across all time points, MIC values for all strains remained within ± 1 twofold dilution of those obtained from freshly prepared microdilution plates (Table 1). For *A. baumannii*, MICs ranged from 1 to 2 $\mu\text{g/mL}$; for *K. pneumoniae*, from 32 to 64 $\mu\text{g/mL}$; and for *P. aeruginosa* ATCC27853, from 0.5 to 1 $\mu\text{g/mL}$. All values remained within one doubling dilution of the initial MIC, indicating no significant loss of colistin activity during 90 days of frozen storage at -20°C .

Table 1 Stability of pre-diluted colistin stored at -20 °C in 96-well microdilution plates determined by MIC values of test strains over 90 days

Days	MIC (µg/mL) ^a								
	<i>A. baumannii</i> (Clinical isolate)			<i>K. pneumoniae</i> (Clinical isolate)			<i>P. aeruginosa</i> ATCC27853 (0.25 - 2 µg/mL) ^b		
	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3	Rep1	Rep2	Rep3
0	1/1	1/1	1/1	64/64	64/64	64/64	0.5/0.5	0.5/0.5	0.5/0.5
15	1/1	1/1	2/2	32/32	32/32	64/64	1/1	1/1	1/1
30	1/1	2/2	2/2	32/32	32/32	64/64	1/1	0.5/0.5	1/1
45	2/2	1/1	2/2	64/64	32/32	64/64	1/1	1/1	1/1
60	1/1	1/1	1/1	64/64	64/64	64/64	1/1	1/1	0.5/1
75	2/2	1/1	1/1	64/64	64/64	64/64	1/1	1/1	1/1
90	1/1	1/1	2/2	64/64	64/64	64/64	1/1	1/1	1/1

Note: ^a MIC values represent results from three independent biological replicate (Rep1 - Rep3). Value are expressed as X/X, indicating results from technical duplicate wells within the same experiment.

^b Quality Control range for *P. aeruginosa* ATCC27853 according to CLSI M100 guidelines, 2025⁽¹⁰⁾.

Discussion

Colistin is increasingly used to treat infections caused by multidrug-resistant (MDR) gram-negative bacteria, and accurate antimicrobial susceptibility testing (AST) is therefore critically important. The broth microdilution (BMD) method remains the reference procedure recommended by CLSI⁽¹⁰⁾ and EUCAST⁽¹¹⁾ for determining colistin MICs. However, routine implementation of BMD in clinical laboratories can be challenging, as the method is labor-intensive, technically demanding, and susceptible to variations introduced by media preparation, antibiotic dilution accuracy, and staff expertise. Developing a practical approach that allows laboratories to prepare and store pre-diluted colistin microdilution plates in advance would greatly enhance testing efficiency and standardization.

In this study, we demonstrated that pre-diluted colistin stored at -20 °C in 96-well microdilution plates retained its antimicrobial activity for at least 90 days, with MIC values remaining within one twofold dilution of those obtained from freshly prepared plates. Furthermore, this was confirmed by repeating the BMDs using freshly

prepared new drug lots tested on the same day as the stored plates. Minor fluctuations in MIC values were observed among the tested strains during frozen storage; however, these variations did not exceed one twofold dilution, which is within the acceptable range of variability for the BMD method⁽¹⁷⁾. Importantly, interpretive categories remained unchanged throughout the 90-day period, *A. baumannii* remained colistin-intermediate (≤ 2 µg/mL), *K. pneumoniae* remained colistin-resistant (≥ 4 µg/mL), and *P. aeruginosa* ATCC 27853 consistently fell within the established quality-control (QC) range (0.25 - 2 µg/mL). These results indicate that the antimicrobial activity of colistin was maintained during frozen storage, confirming the stability of colistin under these conditions and supporting the feasibility of preparing ready-to-use BMD trays for routine testing.

While our study validated stability across intermediate and resistant MIC ranges, isolates with MICs at or near the clinical breakpoint (2 - 4 µg/mL), such as *mcr-1*-carrying *Escherichia coli*, were not included. Because these borderline isolates are more susceptible to minor MIC shifts,

further investigation is warranted to fully confirm the robustness of pre-diluted plates for detecting low-level resistance. Future studies specifically designed to assess breakpoint robustness and limits of detection for emerging resistance mechanisms will be necessary.

Our findings are consistent with previous investigations of antibiotic stability under frozen conditions. Healan et al. reported that reconstituted colistin methanesulfonate remained stable for up to 24 hours at temperatures ranging from 21 °C to -70 °C, whereas Li et al. demonstrated that colistin was stable in aqueous solution at 4 °C and 37 °C for up to 60 days and 120 hours, respectively^(18,19). Rogacka et al. found that colistin-containing agar plates stored at 4 °C for seven days showed no change in MIC, and earlier studies reported-remarkable thermal stability of colistin at 121 °C⁽²⁰⁻²²⁾. Similar results have been documented for other antibiotics classes: Leite et al. showed that six β -lactams (meropenem, ertapenem, imipenem, ceftriaxone, ceftazidime, and piperacillin-tazobactam) could be frozen as a stock concentration for up to six months at -80 °C without a significant loss of activity⁽¹³⁾; Nickolai et al. demonstrated that 11 β -lactams (ampicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, cefazolin, cefotaxime, moxalactam, cefoperazone, ceftriaxone, and imipenem) remained stable for 52 weeks at -70 °C⁽²³⁾; and Okerman et al. reported that tetracyclines and quinolones could be stored for six months at -20 °C⁽²⁴⁾. Collectively, these findings support that freezing effectively preserves antibiotic potency.

The observed stability of colistin in this study may be attributed to its chemical structure. Colistin sulfate is a cationic polypeptide that undergoes hydrolysis in aqueous solutions, a process known to be temperature-dependent⁽¹⁾. Lower storage temperatures slow hydrolytic degradation, thereby preserving structural integrity and antimicrobial activity of the compound⁽²⁵⁾. Storage at -20 °C therefore likely minimizes hydrolytic breakdown, ensuring stability for extended periods compared with storage at higher temperatures.

Most previous stability studies assessed concentrated antibiotic stock solutions that were later diluted for testing, whereas the present work specifically examined colistin that had already been diluted and distributed into microdilution trays. This approach more closely reflects real-world laboratory practice and demonstrates that pre-diluted trays remain reliable for at least three months. Frozen BMD plates can therefore be prepared in bulk, stored, and used as needed, requiring only the addition of bacterial inoculum before incubation. This strategy reduces preparation time, minimizes handling errors, and enhances inter-laboratory standardization.

Overall, this study provides new experimental evidence that colistin retains its antimicrobial activity when stored at -20 °C in a pre-diluted 96-well microdilution plate format for at least 90 days. The ability to prepare and store frozen BMD trays offers a simple, standardized, and cost-effective solution for clinical laboratories, facilitating accurate and reproducible colistin susceptibility testing in accordance with CLSI and EUCAST guidelines.

Although the number of bacterial strains evaluated was limited and the study was not designed as a full test-performance verification, the findings support the feasibility of this approach. Future studies incorporating larger numbers of clinical isolates representing intermediate, and resistant profiles, as well as isolates with MICs near the clinical breakpoint (2 - 4 $\mu\text{g/mL}$), will be necessary to further validate its applicability in routine clinical laboratories.

Conclusion

This study demonstrated that diluted colistin can be stored as a working solution at -20 °C for at least three months without loss of antimicrobial activity. The proposed approach provides a practical and user-friendly procedure for preparing pre-diluted colistin trays in advance, saving time and reducing dilution-related handling errors by laboratory personnel, thereby supporting

consistent and reproducible MIC determinations. However, verification of the biological activity of stored plates after three months of storage is recommended to ensure continued reliability.

Take home messages

This study demonstrates that pre-diluted colistin stored at -20 °C in 96-well microdilution plates remains stable and retains full antimicrobial activity for at least 90 days. These findings provide practical evidence that frozen, ready-to-use BMD plates can be reliably used for colistin susceptibility testing, reducing preparation time and variability.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Aschana Tirapattanuna: Data curation, Formal analysis, Investigation, Validation, Visualization, Writing - Original draft.

Pintip Suchartlikitwong: Writing - Review & Editing.

Sunisa Chirakul: Conceptualization, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Formal analysis, Writing - Review & Editing.

Data availability

Data available on request from the authors.

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Immediate effects of kinesiology tape on pain and quadriceps muscle strength in individuals with patellofemoral pain syndrome

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KEYWORDS

Anterior knee pain;
Patella pain;
Squatting;
Lower extremity
function;
Muscle strength.

ABSTRACT

Patellofemoral pain syndrome (PFPS) is a common condition causing anterior knee pain during performing activities, such as squatting, running, and stair climbing. A kinesiology tape (KT) is often used to reduce pain and improve muscle functions, but its immediate effects remain unclear. This study investigated immediate effects of KT on pain and muscle strength in individuals with PFPS. Thirty-two male participants diagnosed with PFPS from Thailand National Sports University, Samut Sakhon campus, were recruited and randomly assigned into two groups using a simple randomization method. The placebo group ($n = 16$; age 20.0 ± 1.4 years) received I-shaped KT applied 7 cm above and below the patella without tension. The intervention group ($n = 16$; age 20.2 ± 1.4 years) received KT using a Y- and I-shaped application technique for quadriceps facilitation and patellar correction. Outcomes were measured before and immediately after KT application, including pain intensity assessed using the visual analog scale (VAS), squat range of motion (ROM) measured by goniometer, and quadriceps muscle strength measured using a hand-held dynamometer. There were no significant differences between the groups at baseline. Within-group analysis showed that the placebo group demonstrated significant improvements in pain (p -value = 0.002) and muscle strength (p -value = 0.012). In the intervention group, significant improvements were observed in pain (p -value = 0.001), squat ROM (p -value = 0.001), and muscle strength (p -value = 0.005) after KT application. However, no significant differences were found between the intervention and placebo groups in post-test outcomes (p -value > 0.05). KT may be used as an alternative treatment option to reduce pain, and improve squat ROM and quadriceps muscle strength in individuals with PFPS. However, the immediate outcomes were not significantly better than the placebo taping applications.

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Introduction

Patellofemoral pain syndrome (PFPS) is one of the most common musculoskeletal disorders and is often referred to as anterior knee pain. Patients with PFPS typically report pain and discomfort in the anterior aspect of the knee, particularly around the medial border of the patella^(1,2). The symptoms are commonly aggravated during performing functional activities that increase stress on the patellofemoral joint, such as running, ascending and descending stairs, squatting, jumping, or prolonged sitting with the knee flexed⁽¹⁻³⁾.

The exact cause of PFPS remains unclear; however, it has been associated with abnormal lateral tracking of the patella⁽¹⁾. Several factors may also contribute to PFPS, including tightness of the iliotibial band⁽²⁾, muscular weakness, patellar malalignment, and neuromuscular dysfunction⁽³⁾. In addition, abnormal patellar positioning due to an imbalance of muscular forces acting on the patella may result in altered patellar tracking. The vastus medialis oblique (VMO) and vastus lateralis (VL) muscles are important dynamic stabilizers of the patella, particularly during the final 30 degrees of knee extension. Therefore, weakness of the VMO has been suggested as an additional contributing factor to abnormal patellar tracking in individuals with PFPS⁽¹⁾.

Conventional treatments for PFPS include stretching and strengthening exercises for the muscles surrounding the knee joint, activity modification, therapeutic ultrasound, thermotherapy, cryotherapy, knee bracing, and patellar taping. The primary goals of these treatments are to restore normal patellofemoral joint biomechanics, reduce joint stress, and improve quadriceps muscle strength^(2,4). Recently, a kinesiology tape (KT), developed in the late 1970s by Dr. Kenzo Kase and colleagues in Japan⁽⁵⁾, has become increasingly popular as an adjunct treatment for PFPS. This elastic therapeutic tape contains embedded elastic fibers that allow it to stretch and recoil when applied to the skin. KT

also allows a relatively full range of joint motion while providing support to the underlying tissues. Some characteristics of KT include its thickness, which is similar to that of human skin, its ability to stretch up to approximately 120%-140% of its original length, and its ability to remain applied to the skin for up to 3-4 days^(6,7).

Although KT has been widely used in clinical practice for the management of PFPS, previous studies have reported inconsistent findings regarding its effectiveness in reducing pain and improving muscle function^(8,9). In addition, most previous studies have focused on functional outcomes or kinematic changes, while limited research has specifically examined the immediate effects of KT on pain, squat range of motion (ROM), and quadriceps muscle strength in individuals with PFPS. Furthermore, evidence regarding the short-term neuromuscular effects of KT in young adults with PFPS remains limited. Therefore, the purpose of this study was to investigate the immediate effects of KT on anterior knee pain, squat ROM, and quadriceps muscle strength in young adults with PFPS.

Materials and Methods

Participants

This randomized controlled trial was conducted in young males diagnosed with PFPS by an experienced musculoskeletal physical therapist from Thai National Sports University, Thailand. The required sample size was calculated using G*Power software based on a two-way ANOVA (group $\times$ time interaction), with an alpha level of 0.05, a statistical power (1- β) of 0.95, and an assumed medium effect size ($f = 0.25$). The calculation indicated that 32 participants were required.

The inclusion criteria were: (1) pain intensity between 3 and 8 on a 10-point pain scale; (2) anterior knee pain during at least two functional activities, including descending stairs, ascending stairs, squatting, running, jumping, or prolonged sitting with the knee flexed at 90°; and

(3) a history of anterior knee pain for at least six months ⁽¹⁻³⁾.

Participants were excluded if they had a history of knee trauma, previous knee surgery (except arthroscopy), meniscopathy, ligament injury, knee joint effusion, knee osteoarthritis, or patellar subluxation or dislocation.

This study was approved by the Human Ethics Committee of Huachiew Chalermprakiet University (Approval No. A.898/2562). All participants were informed about the study procedures and provided written informed consent prior to participation.

A total of 32 participants were randomly assigned to two groups (1:1) using a computer-generated random number sequence (Figure 1). Due to the nature of the intervention, blinding of participants and therapists was not feasible. However, outcome assessment was performed by a blinded assessor who was not involved in group allocation at baseline and immediately after the intervention. The placebo taping was applied in a similar manner to the intervention but without tension or therapeutic effect.

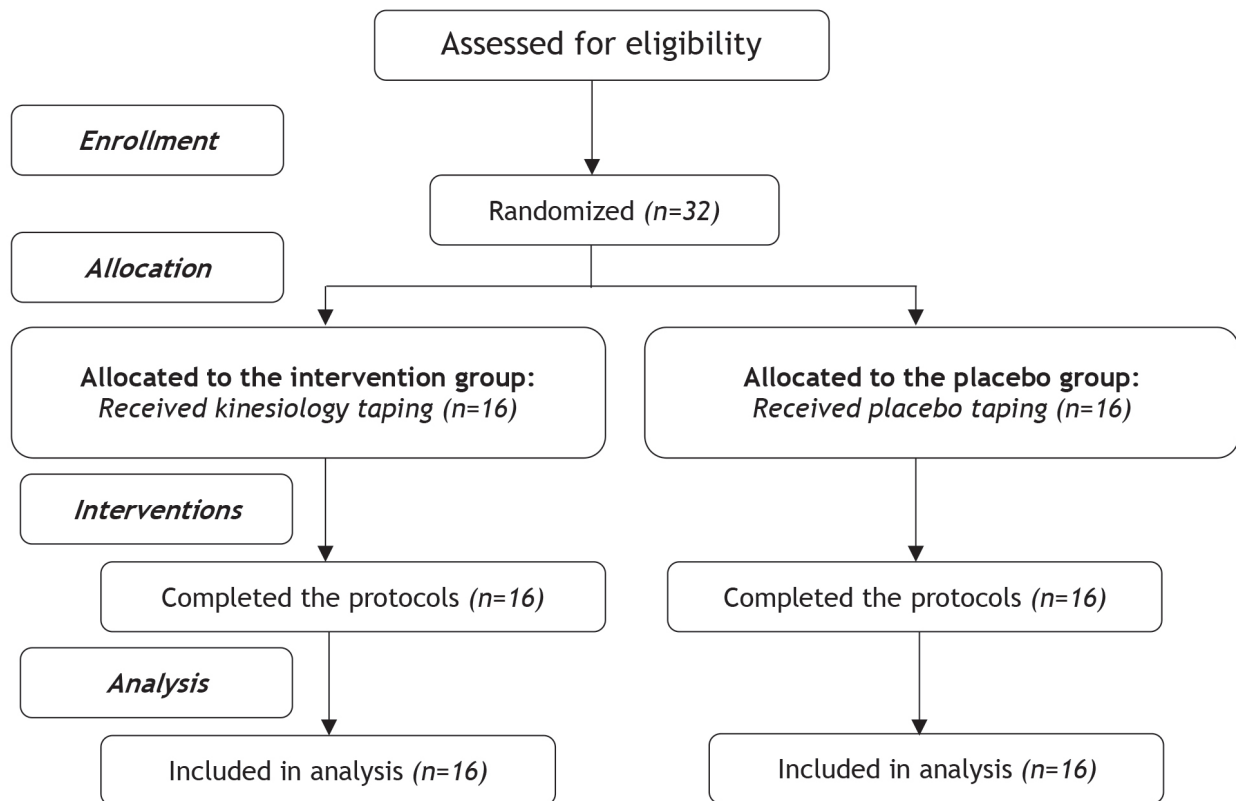


Figure 1 CONSORT flow diagram of participant recruitment, allocation, intervention, and analysis

Taping methods

Intervention group: Participants received KT consisting of VMO facilitation and patellar correction techniques. With the knee flexion to approximately 90°, a Y-shaped KT was applied to

the quadriceps muscle. The anchor of the tape was placed at the mid-thigh with no tension. Each tail of the Y-strip was applied along the quadriceps muscle, bracketing the patella and terminating below it. The middle portion of the

tape was applied with moderate tension, whereas the tails were applied without tension. In addition, a patellar correction I-strip was applied with the knee flexed at approximately 90° , using moderate tension along the medial aspect of the patella⁽¹⁰⁾ as shown in Figure 2a and 2b.

Control group: Participants received placebo KT to avoid any mechanical influence

on patellar alignment. Two I-strips were applied horizontally with the knee in a neutral position. One strip was placed approximately 7 cm above the superior border of the patella, and the second strip was placed approximately 7 cm below the inferior border of the patella. Both strips were applied without tension⁽¹⁰⁾ as shown in Figure 2c.

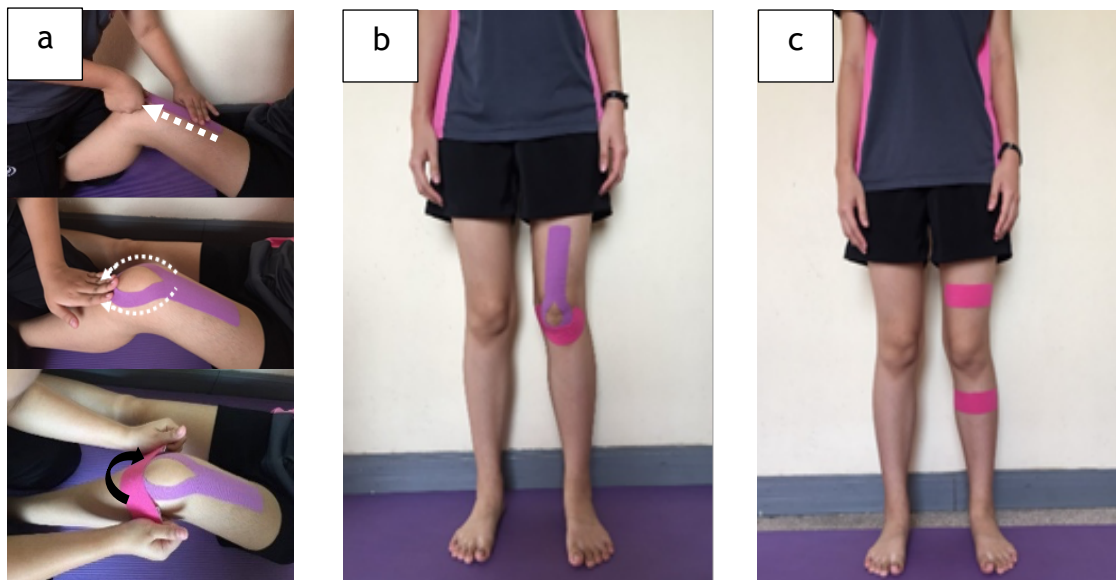


Figure 2 Kinesiology tape (KT) application for the participants
(a and b) Y-I shape KT application for the intervention group
(c) Placebo KT application (I-strips without tension) for the control group

The KT tape procedures of each group were delivered by a trained physical therapist. All participants received the allocated intervention as intended, with no protocol deviations. Participant adherence was complete, as all participants underwent the taping procedure and completed all outcome assessments (Figure 1). No concomitant care or additional interventions were provided to participants in either group during the study period. Participants were monitored during and immediately after the interventions and were asked to report any discomfort, pain, or skin irritation related to the KT used.

Outcome measures

The primary outcome of this study was pain intensity, assessed using the visual analog scale (VAS). Participants were instructed to indicate their level of pain on a 10-cm horizontal line, where 0 represented “no pain” and 10 represented “worst imaginable pain,” during a squatting task⁽⁹⁾.

Secondary outcomes included squat ROM and quadriceps muscle strength. Details of the tests are as follows.

- Knee flexion ROM during squatting was measured using a universal goniometer. Participants were instructed to stand and perform a squat

to the point of pain, at which the knee flexion angle was measured ^(9,10).

- Quadriceps muscle strength was assessed using a hand-held dynamometer with a curved transducer to measure isometric knee extensor strength (Figure 3). Participants were positioned in a supine position with the affected knee in flexion approximately 90° on a quadricep board. The dynamometer

was placed approximately 5 cm proximal to the lateral malleolus. Participants were instructed to perform maximal voluntary isometric contraction, and verbal encouragement was provided. Three trials were performed, with a 3-minute rest between trials to minimize fatigue, and the average value was used for analysis ⁽¹⁵⁾.



Figure 3 Quadriceps muscle strength was measured through a hand-held dynamometer.

Statistical analysis

As the data were normally distributed according to the Shapiro-Wilk test, all data were presented using the mean $\pm$ standard deviation (SD). A two-way mixed analysis of variance (two-way ANOVA) was performed to examine differences between the groups (intervention vs. control) and conditions (pre and post-intervention). Given that the outcomes were assessed immediately, the analysis focused primarily on group differences and condition-related changes rather than long-term time effects. Statistical significance was set at p -value < 0.05 .

Results

Participant characteristics

Thirty-two young male participants with average age of approximately 20 years old completed the study. The participant characteristics, including age and body mass index, and baseline symptom severity, which was classified as mild to moderate, were comparable between the two groups (Table 1, p -value > 0.05).

Table 1 Participant characteristics (16 participants/group).

Variable	Control		Intervention	
	Mean	SD	Mean	SD
Age (year)	20.00	1.37	20.19	1.42
Weight (kg)	68.20	12.97	62.8	11.63
Height (cm)	173.80	6.30	169.70	7.80
BMI (kg/m ²)	22.36	3.39	21.72	3.14

The baseline data indicated no significant differences for all outcomes of the study (Table 2). After the treatments, participants in both groups showed significant reduction in pain scores, with increased quadriceps muscle strength (p -value < 0.05). However, the significant improvement in

squat ROM was found only in the KT group (p -value = 0.001, Table 2). The data comparisons after treatments, however, suggested no significant differences between the groups (p -value > 0.05). Participants in both groups additionally reported no adverse events of using the KT.

Table 2 Comparison of pain intensity and quadriceps muscle strength between the kinesiology taping and placebo groups before and after intervention using two-way ANOVA

Variable	Control (mean $\pm$ SD)	Intervention (mean $\pm$ SD)	Mean difference	p -value ^a
Visual analog scale (cm)				
Before	3.53 $\pm$ 1.56	4.16 $\pm$ 1.37	-1.59 to 0.31	0.181
After	2.64 $\pm$ 1.44	2.02 $\pm$ 1.73	-5.22 to 1.77	0.275
p -value ^b	0.002*	0.001*		
Squat range of motion (degree)				
Before	67.69 $\pm$ 19.68	64.88 $\pm$ 19.32	-11.27 to 16.90	0.686
After	72.69 $\pm$ 15.30	79.00 $\pm$ 14.85	-17.20 to 4.58	0.246
p -value ^b	0.131	0.001*		
Quadriceps muscle strength (kg)				
Before	22.44 $\pm$ 3.56	22.39 $\pm$ 3.42	-2.47 to 2.57	0.967
After	24.01 $\pm$ 2.56	24.18 $\pm$ 2.20	-1.89 to 1.56	0.844
p -value ^b	0.012*	0.005*		

Note: ^a p -value from two-way mixed analysis of variance (two-way ANOVA), ^b p -value from dependent samples t-test. *indicate significant differences.

Discussion

The present study investigated the immediate effects of KT on pain intensity, squat ROM, and quadriceps muscle strength in young adults with PFPS. The findings demonstrated significant improvements in pain and muscle strength in both groups, while squat ROM improved only in the intervention group. However, data comparisons showed no significant differences between the groups in all variables (p -value > 0.05, Table 2).

The reduction in pain observed in this study may be explained by the neurophysiological mechanisms associated with KT. The elastic

properties of the tape are believed to stimulate cutaneous mechanoreceptors⁽⁷⁾, which may modulate nociceptive input through the pain gate mechanism⁽⁵⁾. This sensory stimulation may alter afferent feedback and subsequently reduce perceived pain during functional activities such as squatting^(8,9). Additionally, the mechanical lifting effect of the tape on the skin may improve local circulation and reduce pressure on pain-sensitive structures around the patellofemoral joint^(8,12). The pain improvement observed in control group, may be due to the effects of tactile stimulation provided by the tape itself, a placebo response, natural pain fluctuation, or regression to the

mean. The physical presence of any tape on the skin may generate sufficient cutaneous afferent input to partially modulate nociception, even in the absence of therapeutic tension^(5,7). However, the lack of significant differences between groups in the present study is consistent with previous studies reporting that KT does not demonstrate superior effects compared with placebo or other conservative interventions in individuals with PFPS^(8,9,12).

Improvements in quadriceps muscle strength were observed in both groups, while squat ROM improved significantly only in the intervention group. These findings may be associated with reduced pain inhibition and improved neuromuscular activation during functional tasks^(8,13). The improvement in quadriceps muscle strength following KT may be related to enhanced neuromuscular activation of the quadriceps muscle, particularly the VMO⁽¹³⁻¹⁵⁾. Previous studies have suggested that KT may facilitate muscle activation by improving proprioceptive feedback and optimizing muscle recruitment patterns⁽¹⁶⁾. Increased sensory input from the tape may enhance motor unit recruitment and improve muscle performance during isometric contraction^(13,15). However, the effects are often small and not significantly different from control conditions. This supports the findings of the present study, indicating that the benefits of KT may be related to psychological or proprioceptive mechanisms rather than direct mechanical effects⁽¹⁴⁾. Importantly, no adverse events or unintended effects were reported in either group, indicating that KT is a safe intervention for individuals with PFPS when applied appropriately. This is consistent with previous literature reporting minimal risk associated with taping interventions^(5,8,14).

The present findings extend existing literature by specifically examining the immediate effects of KT on pain, squat ROM, and quadriceps muscle strength simultaneously in young male athletes with PFPS - a population that has

received limited attention in previous studies. While earlier research has primarily focused on functional outcomes or kinematics over longer durations, the present study addresses the gap in evidence regarding acute neuromuscular responses to KT. The results are consistent with previous research reporting beneficial effects of KT in individuals with PFPS. Several studies have demonstrated that KT can reduce pain and improve knee function by assisting patellar alignment and enhancing neuromuscular control^(14,16). These results support the clinical application of KT as an adjunct intervention for managing symptoms associated with PFPS⁽¹⁶⁾.

However, some previous studies have reported inconsistent findings regarding the effectiveness of KT. Differences in taping techniques, participant characteristics, and outcome measurements may contribute to these variations. In the present study, a combined approach of VMO facilitation and patellar correction was applied. This may have contributed to the observed improvements in pain and quadriceps muscle strength through enhanced neuromuscular activation of VMO and improved patellar tracking, thereby optimizing patellofemoral joint mechanics^(13,15). These findings suggest that the immediate effects of KT may be influenced by the specific application technique used, which could explain differences compared with previous literature.

From a clinical perspective, therefore, KT may provide an immediate therapeutic effect for individuals with PFPS. This intervention may be particularly useful during rehabilitation programs to reduce pain and facilitate quadriceps muscle activation during exercise or functional training.

Study limitations

Several limitations should be noted. This study included only male participations from a single institution, which limits the generalizability of the findings to other populations. The study was also limited to young athletes, so results may not be applied to older or less active individuals with PFPS. The short study duration assessed only

immediate effects, and no long-term follow-up was conducted to evaluate sustained outcomes of KT in individuals with PFPS. Additionally, the use of a hand-held dynamometer rather than an isokinetic dynamometer may limit the precision of muscle strength measurements. Future studies should examine the effects of KT using electromyography and biomechanical analysis to further elucidate the neuromuscular mechanisms underlying the immediate responses to taping. Research including female participants, older adults, and diverse clinical settings is also warranted to improve generalizability of findings.

Conclusions

KT demonstrated significant immediate effects in reducing pain intensity and improving quadriceps muscle strength in individuals with PFPS. The present findings suggest that KT may enhance neuromuscular activation of the quadriceps muscles and provide short-term pain relief during functional activities. Therefore, KT may be considered as an effective adjunct intervention in the rehabilitation of individuals with PFPS.

Take home messages

KT may help to reduce pain, improve quadriceps muscle function, and improve squat ROM in young male individuals with PFPS, making it a beneficial adjunct to support rehabilitation and functional activities. However, the immediate benefits of KT were not significantly superior to placebo taping, suggesting that psychological and proprioceptive mechanisms may contribute to its effects.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Nipaporn Rigo: Conceptualization, Methodology, Formal analysis, Resources, Funding acquisition, Writing - Original draft.

Atcharaphan Changkeaw: Conceptualization, Methodology, Formal analysis, Writing - Review & Editing.

Data availability

The datasets analyzed during the current study are not publicly available due to ethical and privacy restrictions to protect participant confidentiality but are available from the corresponding author on reasonable request.

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Effects of aerobic and combined exercise interventions on CD4 cell counts and VO_2 max in people living with HIV: a systematic review and meta-analysis

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KEYWORDS

Aerobic exercise;
Cardiorespiratory
fitness;
Immune function;
Non-pharmacological
treatment; Physical
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ABSTRACT

Exercise has been proposed as a non-pharmacological strategy to improve immune functions and cardiorespiratory fitness in people living with human immunodeficiency virus (HIV). This systematic review and meta-analysis evaluated the effects of aerobic and combined exercise interventions on the cluster of differentiation 4 (CD4) cell counts and maximal oxygen consumption (VO_2 max). Randomized controlled trials were identified and pooled using random-effects models. Seven trials comprising 366 participants were included. Meta-analysis demonstrated a significant improvement in VO_2 max following exercise interventions compared with control conditions, while a favorable but non-significant effect was observed for CD4 cell counts. Several included studies implemented combined exercise modalities (e.g., aerobic with resistance training), which may have contributed to the observed effects. These findings suggest that exercise interventions, particularly those including aerobic components, improve cardiorespiratory fitness and may provide modest immunological benefits in people living with HIV. Further adequately powered trials are required to clarify the independent effects of different exercise modalities and to determine optimal exercise prescriptions.

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Introduction

Human immunodeficiency virus (HIV) infection remains a significant global public health challenge, with an estimated 38 million people living with HIV worldwide in 2022⁽¹⁾. The widespread implementation of antiretroviral therapy (ART) has dramatically improved life expectancy and reduced the incidence of opportunistic infections among people living with HIV. However, despite effective viral suppression, many patients continue to experience persistent immunological dysfunction, chronic inflammation, and comorbidities, such as cardiovascular disease, pulmonary complications, and metabolic disorders^(2,3). These factors collectively contribute to reduced physical functions and quality of life, creating a pressing need for comprehensive management strategies beyond pharmacological treatments.

Reduced CD4 cell counts are associated with impaired immune surveillance and increased susceptibility to opportunistic infections⁽⁴⁾. In addition, chronic inflammation, mitochondrial dysfunction, and muscle wasting in individuals living with HIV contribute to reduced aerobic capacity, increased fatigue, and diminished physical function⁽⁵⁻⁶⁾. These physiological alterations negatively impact quality of life and increase the risk of comorbidities⁽⁷⁾.

Aerobic exercise has emerged as a promising non-pharmacological intervention to address these challenges by improving cardiovascular endurance, muscle strength, and immune function⁽⁸⁾. Numerous studies have shown that regular aerobic exercise can enhance VO_2 max and improve quality of life in HIV-infected populations⁽⁹⁻¹¹⁾. Moreover, some evidence suggests that aerobic exercise may help stabilize or increase CD4 cell counts, potentially mitigating immune decline⁽¹²⁾. Despite this, results across studies have been inconsistent, and optimal exercise prescriptions including intensity, frequency, and duration, have yet to be standardized for this population.

Although previous systematic reviews have examined the role of exercise in HIV populations,

most have evaluated exercise interventions broadly without clearly distinguishing between aerobic and non-aerobic modalities. Furthermore, many studies implement combined exercise programs, making it difficult to isolate the independent effects of specific exercise types. Therefore, this study aims to systematically evaluate the effects of aerobic and combined exercise interventions on CD4 cell counts and VO_2 max in people living with HIV. The findings will contribute to evidence-based recommendations for incorporating aerobic exercise into comprehensive HIV management, ultimately aiming to improve patients' health outcomes and quality of life.

Materials and methods

Definition of exercise modalities

Aerobic exercise was defined as continuous, rhythmic physical activity aimed at improving cardiorespiratory endurance (e.g., walking, cycling, treadmill exercise). Non-aerobic exercise referred to exercise modalities that primarily target on strength or anaerobic performance, including resistance or strength training. In studies involving combined exercise interventions (e.g., aerobic plus resistance training), exercise classification was based on the predominant component of the intervention, considering the primary training objective, proportion of session time, and protocol description reported in the original studies.

Data sources and search strategy

Search Strategy

A comprehensive search was conducted in MEDLINE, SCOPUS, EMBASE, IPA, CINAHL, and Cochrane Library databases from inception to January 2023. The search strategy utilized a combination of Medical Subject Headings (MeSH) and relevant keywords, including "aerobic exercise", "HIV", "CD4", and " VO_2 max". Boolean operators were applied to combine search terms as follows: ("aerobic exercise" AND "HIV" AND "CD4") and (" VO_2 max").

No language restriction was applied; however, only randomized controlled trials were included. In addition, the reference lists of all included studies and relevant reviews were manually screened to identify any further eligible articles. This systematic review and meta-analysis were conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines⁽¹²⁾. The primary outcomes of interest were CD4 cell counts, as a marker of immune function, and VO₂ max, representing cardiorespiratory exercise capacity in individuals living with HIV.

The search strategy described earlier was used to identify titles and abstracts of studies potentially relevant to the objective of this review. Each abstract was independently screened by two reviewers to assess its eligibility. If at least one reviewer considered a study to be potentially eligible, the full-text article was retrieved for detailed evaluation. Both reviewers then independently reviewed the full text to determine whether it met the predefined inclusion and exclusion criteria. In the event of disagreement, the reviewers discussed their decisions and reached consensus through joint deliberation. Studies were included if they investigated the effects of aerobic exercise interventions in individuals living with HIV and reported outcomes on CD4 cell counts and VO₂ max. Studies were excluded if they were non-original publications such as commentaries, letters, reviews, surveys, or editorials. Duplicate studies using the same population were also excluded, as were studies that did not report effect estimates or lacked sufficient data to calculate the impact of the intervention.

Data extraction was conducted independently by two reviewers using a standardized data extraction form. Extracted information included the first author and year of publication, study design, sample size, participant characteristics (e.g., age, sex, HIV status), details of the exercise intervention (type, frequency, intensity, and duration), comparison or control group (if ap-

plicable), outcome measures (CD4 cell counts and VO₂ max), duration of follow-up, and key findings including effect sizes. Any discrepancies in data extraction were resolved by discussion and, if necessary, consultation with a third reviewer.

The methodological quality of the included studies was assessed using the Physiotherapy Evidence Database (PEDro) scale, which evaluates internal validity and statistical reporting. The scale includes 11 items, with a maximum score of 10 (excluding the first item, which addresses external validity). Each study was independently assessed by two reviewers. Studies were classified as high quality (scores of 6-10), moderate quality (scores of 4-5), or low quality (scores below 4). Discrepancies in scoring were resolved through discussion to reach a consensus. Although studies with low quality were not excluded, their limitations were considered during the interpretation of the overall results.

Study selection

The search strategy outlined earlier was applied to identify titles and abstracts of studies potentially relevant to this review. Each abstract was independently assessed by two reviewers. If at least one reviewer considered a study to be potentially eligible, the full-text article was retrieved for comprehensive evaluation. Both reviewers independently assessed the full-text articles to determine their eligibility based on predefined inclusion and exclusion criteria. In cases of disagreement, the reviewers discussed the rationale for their decisions and reached a consensus.

The inclusion criteria were as follows: (1) studies that implemented aerobic exercise interventions in individuals living with HIV; and (2) studies that reported outcomes for both CD4 cell counts and VO₂ max. Exclusion criteria included non-original publications such as commentaries, letters, reviews, surveys, or editorials. Additionally, duplicate publications based on the same population, studies without reported effect estimates, or those with insufficient data to

calculate the impact of interventions were excluded.

Data synthesis and analysis

The risk of bias in the included studies was independently evaluated by two reviewers using the Cochrane Collaboration's Risk of Bias tool⁽¹²⁾. This tool assesses methodological quality based on key criteria such as random sequence generation, allocation concealment, blinding, completeness of outcome data, selective reporting, and other potential sources of bias. Any disagreements were resolved through discussion to reach consensus. A meta-analysis was conducted using CD4 cell counts and VO₂max as the primary outcomes. Heterogeneity among studies was assessed using Cochran's Q statistic, with a *p-value* < 0.10 indicating statistically significant heterogeneity. The I² statistic was also calculated to quantify the proportion of total variation across studies that was due to heterogeneity rather than chance. Values of approximately 25%, 50%, and 75% were interpreted as low, moderate, and high heterogeneity, respectively⁽¹³⁾.

Where substantial heterogeneity was detected, possible sources were explored through subgroup analysis or sensitivity analysis. Publication bias was assessed through visual inspection of

funnel plots and tested statistically using Begg's rank correlation test and Egger's regression asymmetry test. A *p-value* < 0.05 in either test was considered indicative of potential publication bias⁽¹⁴⁾.

Results

Search result and included study characteristics

A total of 1,326 studies were identified as shown in Figure 1. After removing duplicates and screening for relevance, potentially eligible articles were retrieved for full-text review. Ninety-seven articles were excluded at this stage, with fourteen studies failing to meet the predefined inclusion criteria. Three studies assessed the effects of exercise interventions that included aerobic components compared with control or non-aerobic conditions on CD4 cell counts. Four studies evaluated the effects of exercise interventions with aerobic components on VO₂max. Ultimately, seven studies were included in the final analysis and the characteristics of included studies are shown in Table 1. The risk of bias for each included study is presented in Figure 2, with a summary of the overall risk of bias shown in Figure 3.

Table 1 Characteristics of included studies

Author (Year)	Country	Study Design	Participants (n)	Frequency (sessions/week)	Intensity	Time	Type	Control Group	Outcomes Measured	Main Findings
Fillipas et al. (2006)	Australia	RCT	40 HIV men	3	Aerobic exercise 60-80% HRmax and resistance exercise 60-80% 1RM	60 minutes/session 6 months	Aerobic e and resistance exercise	Walking	CD4	↑ CD4
Hand et al. (2008)	USA	Quasi	40 HIV men and women	3	Aerobic exercise 60-70% HRmax and resistance exercise	60 minutes/session 6 weeks	Walking/cycling	Read a book, talk or watch television	VO ₂ max	↑ VO ₂ max
Mutimura et al. (2008)	Rwanda	RCT	100 HIV adults	3	60-70% HRmax	60 minutes/session	Aerobic, stabilization, and strengthening	No exercise	CD4, VO ₂ max	↑ VO ₂ max and CD4
Dolan et al. (2010)	Boston	RCT	40 HIV women	3	Aerobic exercise 60-80% HRmax and resistance exercise 60-80% 1RM	60 minutes/session 16 weeks	Home-based progressive resistance training and aerobic exercise	No exercise	CD4, VO ₂ max	↑ VO ₂ max No significant change in CD4
Ogalha et al. (2011)	Brazil	RCT	63 HIV adults	3	Aerobic exercise 70-80% HRmax	60 minutes/session 24 weeks	Combined aerobic, resisted and stretching exercises	Dietary counseling	CD4, VO ₂ max	↑ VO ₂ max and CD4
Mendes et al. (2013)	USA	RCT	29 HIV men and 51 HIV women	3	Aerobic exercise 60-75% HRmax and resistance exercise 60-80% 1RM	40-60 minutes/session 24 weeks	Combined aerobic e and resistance exercise	No exercise	VO ₂ max	↑ VO ₂ max,
Neto et al. (2016)	Brazil	Quasi	63 HIV adults	3	Aerobic exercise 60-70% HRmax	30-40 minutes/session	Aerobic exercise	No exercise	CD4, VO ₂ max	↑ VO ₂ max and CD4

Abbreviation: CD4, cluster of differentiation 4; VO₂ max, maximal oxygen consumption; HIV, human immunodeficiency virus; ART, antiretroviral therapy.

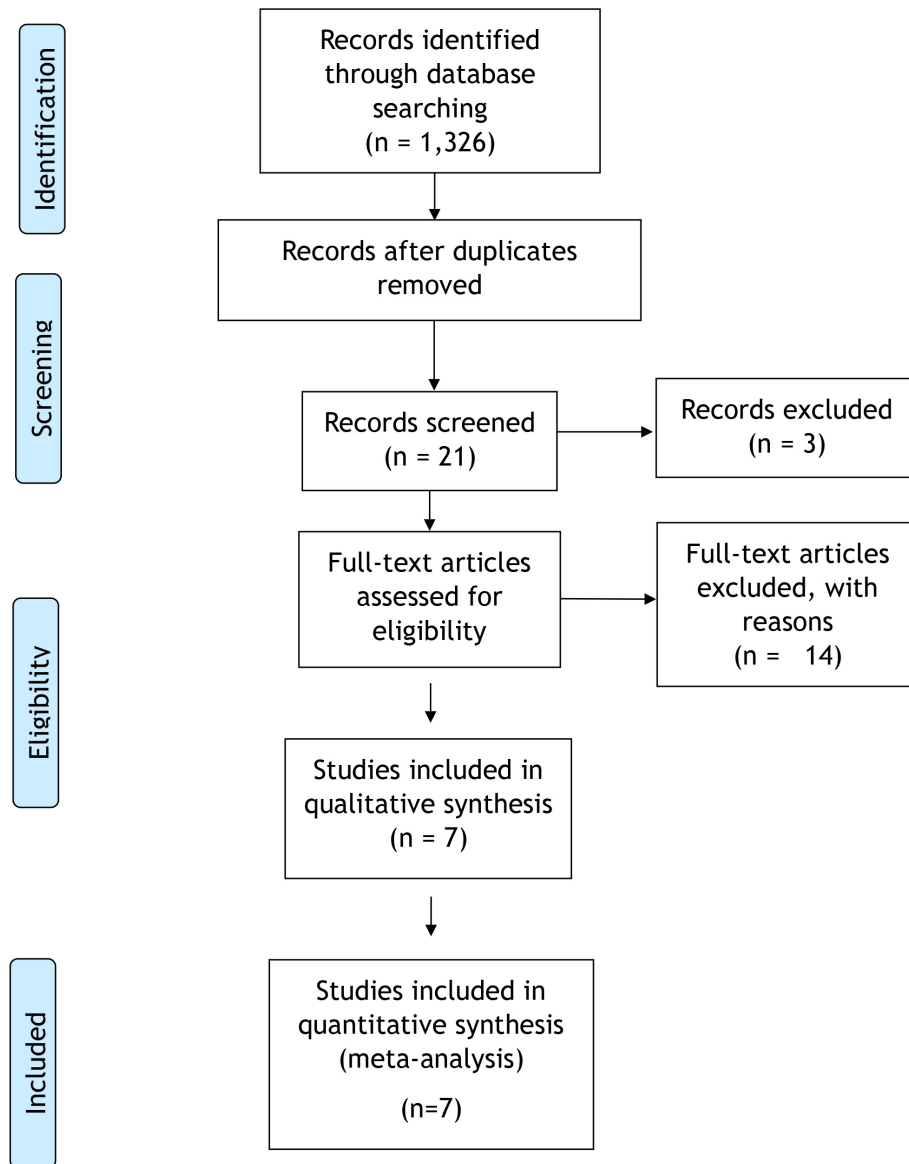


Figure 1 Search and selection of the studies for systematic review and meta-analysis by PRISMA

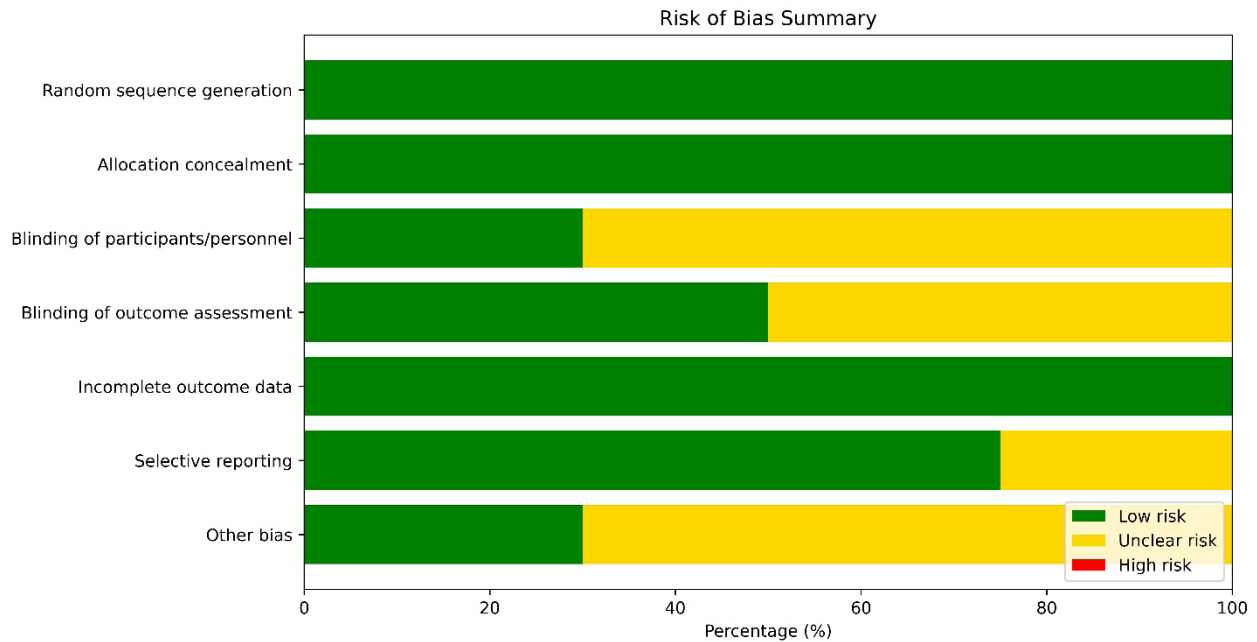


Figure 2 Risk of bias summary presented as percentages across included studies

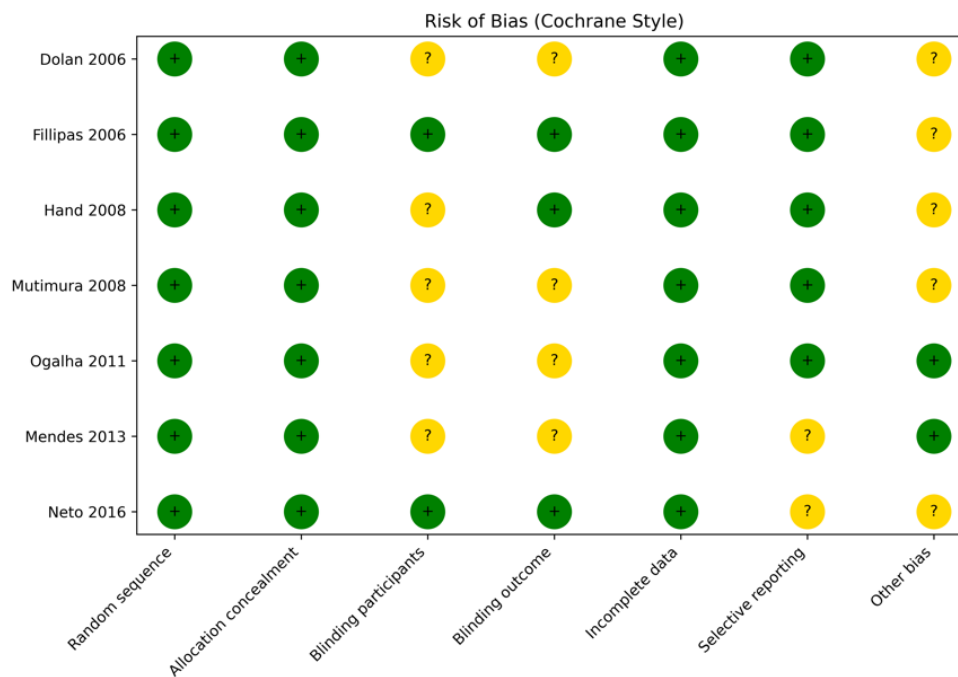


Figure 3 Risk of bias of included studies. Green (+) indicates low risk, yellow (?) unclear risk, and red (-) high risk

Aerobic exercise and CD4 cell count

Three studies assessed the effects of aerobic exercise compared to non-aerobic exercise (control group) on CD4 cell counts⁽⁹⁻¹¹⁾. There was no evidence of heterogeneity among these studies ($I^2 = 0.0\%$; p -value = 0.854), as shown in Figure 4. Funnel plot analyses indicated no apparent publication bias (Egger's test p -value

= 0.117; Begg's test p -value = 0.317). The pooled results showed that participants in the aerobic exercise group had a greater improvement in CD4 cell counts compared to the non-aerobic group, although this difference was not statistically significant (mean difference = -2.92 cells/mm³; 95% confidence interval: -12.56 to 6.73).

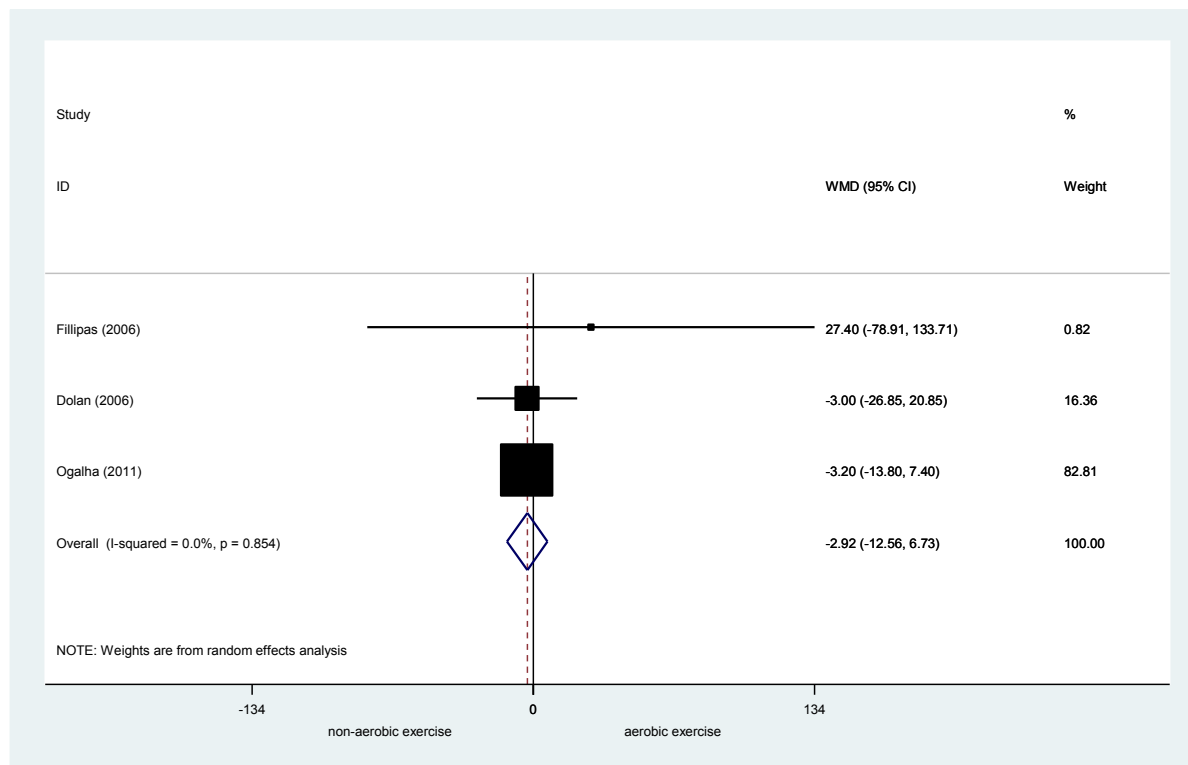


Figure 4 Forest plot showing the effects of exercise interventions on CD4 cell counts in people living with HIV

Aerobic exercise and exercise capacity

Four studies evaluated the effects of aerobic exercise versus non-aerobic exercise on $VO_2\text{max}$ ^(10,12,14). Moderate heterogeneity was observed among these studies ($I^2 = 43.1\%$; p -value=0.153), as illustrated in Figure 5. Funnel plots did not reveal any significant publication

bias (Egger's test p -value=1.00; Begg's test p -value=0.3154). The meta-analysis demonstrated that aerobic exercise significantly improved $VO_2\text{max}$ compared to non-aerobic exercise (mean difference=3.68 mL/kg/min; 95% confidence interval: 2.96 to 4.41)

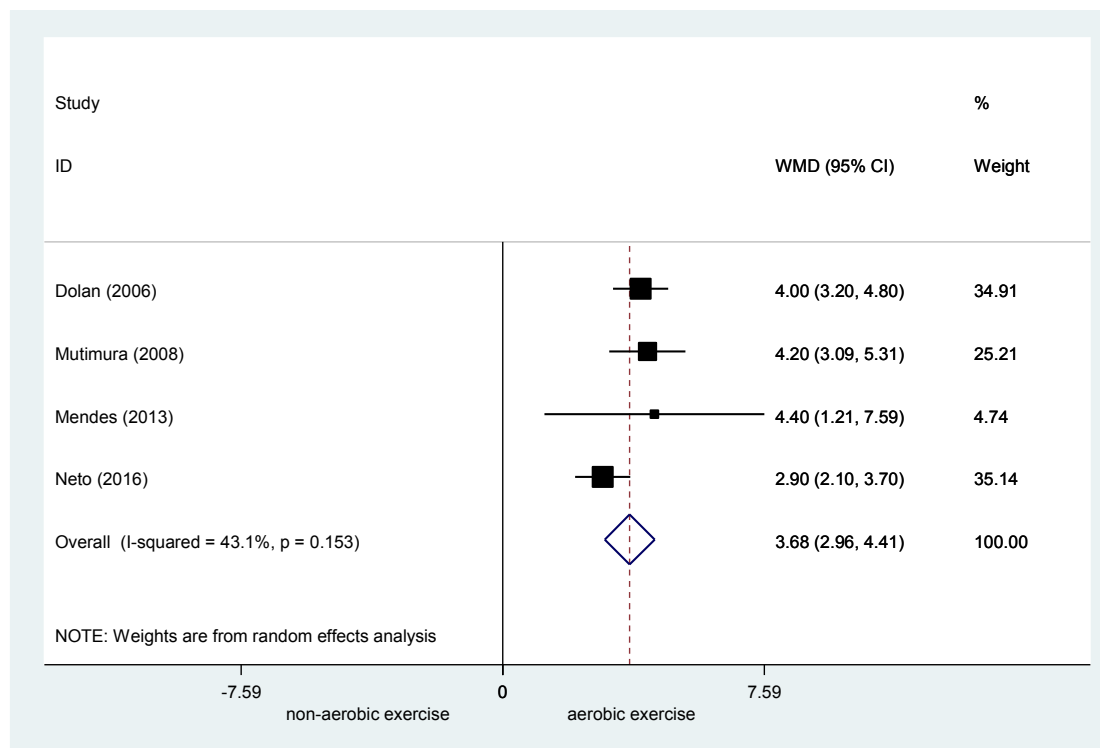


Figure 5 Forest plot showing the effects of exercise interventions on VO₂max in people living with HIV.

Discussion

This meta-analysis evaluated the effects of aerobic exercise on immune function, represented by CD4 cell counts, and cardiorespiratory fitness, measured by VO₂max, in individuals living with HIV. Our findings align with previous studies indicating that aerobic exercise has the potential to enhance physical fitness and may contribute to immunological benefits⁽¹⁵⁾. Although the increase in CD4 cell counts observed was not statistically significant, the trend suggests that regular aerobic exercise could support immune system maintenance or improvement, which is crucial for HIV patients who are often immunocompromised⁽¹⁶⁻¹⁷⁾.

It is important to note that several included studies implemented combined exercise interventions. Therefore, the observed effects cannot be attributed solely to aerobic exercise. The inclusion of mixed exercise modalities may have contributed

synergistically to improvements in both cardiorespiratory fitness and immune function. Exercise may influence immune function through multiple mechanisms, including reductions in systemic inflammation, improved immune cell circulation, and enhanced metabolic regulation. Improvements in VO₂max reflect enhanced cardiovascular efficiency, which is associated with better functional capacity and reduced morbidity in people living with HIV. Additionally, factors such as age, sex, and antiretroviral therapy regimens may influence both CD4 responses and exercise capacity, highlighting the need for individualized exercise prescriptions. Despite promising findings, heterogeneity in intervention protocols and participant characteristics should be considered when interpreting results. The inclusion of combined exercise interventions may have introduced heterogeneity and should be considered when interpreting the findings.

Reduced CD4 cell counts and impaired exercise capacity contribute to physical deconditioning, leading to decreased functional ability and increased risk of secondary complications such as cardiopulmonary disease, metabolic syndrome, and opportunistic infections⁽¹⁸⁻¹⁹⁾. Exercise interventions, particularly aerobic training, are known to mitigate these risks by improving cardiovascular endurance, muscle strength, and overall physical function. Moreover, exercise has psychological benefits, including reduced fatigue and improved mood, which further enhance quality of life in HIV populations⁽¹⁵⁾.

Despite these promising findings, the optimal exercise prescription regarding intensity, frequency, duration, and modality remains to be clearly defined. Individual factors such as disease stage, comorbidities, and baseline physical fitness must be considered to tailor safe and effective programs. Future randomized controlled trials with larger sample sizes and longer follow-up periods are needed to establish evidence-based guidelines that can be integrated into routine clinical care.

Limitations of the current meta-analysis include the relatively small number of eligible studies and some variability in study design and intervention protocols. Additionally, heterogeneity in participant characteristics such as age, sex, and antiretroviral therapy regimens could influence the outcomes. There is also a scarcity of studies simultaneously reporting on both immunological and functional outcomes, highlighting the need for more comprehensive research in this area.

Importantly, education and motivation remain critical components for ensuring adherence to exercise programs among people living with HIV. Healthcare providers, including physical therapists, play a vital role in promoting aerobic exercise as a safe, accessible, and effective adjunct therapy that complements pharmacological treatments.

Conclusion

This systematic review and meta-analysis suggest that exercise interventions, particularly those including aerobic components, significantly improve cardiorespiratory fitness and may provide modest benefits for immune function in individuals living with HIV. However, due to the inclusion of combined exercise interventions, the independent effects of aerobic exercise should be interpreted with caution. Further research is required to establish optimal exercise prescription.

Take home messages

Exercise interventions, particularly those including aerobic components, significantly improve cardiorespiratory fitness. Exercise training demonstrates a favorable, although non-significant, effect on CD4 cell counts, suggesting potential immunological benefits. Aerobic exercise should be considered a safe and effective adjunct to standard HIV care.

Conflicts of interest

The authors declare no conflict of interest.

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Author contributions

Sarayoot Mongkol: Conceptualization, methodology, literature search, data curation, formal analysis, risk of bias assessment, interpretation of data, writing-original draft, writing-review and editing.

Chuenpak Saleesing: Supervision, conceptualization, methodology, literature search, writing-review and editing.

Ettipad Jaiyen: Conceptualization, methodology, and literature search

Tichaporn Sookkue: Formal analysis, methodology, interpretation of data, risk of bias assessment, writing-review and editing.

Sukanya Kreeinthong: Supervision, conceptualization, critical revision of the manuscript, project administration, writing-review and editing.

Data availability

All data analyzed in this study are derived from previously published articles and are available within the article and its supplementary materials. No new datasets were generated or analyzed.

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