Vol.5 No.3 September - December 2025

Greater Mekong Subregion Medical Journal (GMSMJ)

School of Medicine
Mae Fah Luang University

ISSN: 2730-3446 (Online)
ISSN: 2821-9112 (Print)

Journal Name	Greater Mekong Subregion Medical Journal
Abbreviation	GMSMJ
ISSN (Online)	2730-3446
ISSN (Print)	2821-9112
Owner	School of Medicine, Mae Fah Luang University
Aims and Scope	Greater Mekong Subregion Medical Journal is an online and printed, peer reviewed international scientific journal published by Mae Fah Luang University. The journal aims to publish articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand, international and especially in countries of Greater Mekong Subregion. Manuscripts submitted to Greater Mekong Subregion Medical Journal will be accepted on the conditions that the author must not have previously submitted that paper to another journal elsewhere. The journal will not charge for any submission. The reproduction or copy of the articles included the pictures should be under the permission of the publisher.
Language	Full text and Abstract in English
Abstracting and Indexing Information	Thai citation index (TCI) and Google scholar
Frequency	3 issues per year (January-April, May-August and September-December)
Editorial office	School of Medicine, Mae Fah Luang University 365 Moo 12 Nanglae Sub District, Muang District, Chiang Rai, THAILAND 57100 Phone: 053-914500 Fax: 053-914505 E-mail: med@mfu.ac.th, apichai.lee@mfu.ac.th Website: http://medicine.mfu.ac.th https://he02.tci-thaijo.org/index.php/gmsmj/
Support Agency	Mae Fah Luang University

Published by:
Tana Press, Phone +6625304114, Fax +6621088950, Website www.tanapress.co.th

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Publication Ethics

Publication ethics of Greater Mekong Subregion Medical Journal (GMSMJ)

Greater Mekong Subregion Medical Journal presents articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand and international, especially in countries of Greater Mekong Subregion.

The journal publishes 3 issues a year: Issue 1 (January - April), Issue 2 (May - August) and Issue 3 (September -December). All submitted research articles and review articles will be evaluated by a single blinded peer-review process and reviewed by 2 experts who have knowledge, expertise, and experience in the field of medicine and related health sciences prior to publication. The journal encloses the information of authors and reviewers. In case of a difference of evaluation, the article evaluation will be considered and given a final decision.

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Original Article

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Greater Mekong Subregion Medical Journal

Incidence of Adjuvant Radiation Therapy after Radical Hysterectomy and Preoperative Prognostic Factors in Cervical Cancer Patients (Stage IA2-IIA1) in Lampang Hospital, Ten Years' Experience

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Received 1 March 2025 • Revised 6 April 2025 • Accepted 6 April 2025 • Published online 1 September 2025

Abstract:

Introduction: For early-stage cervical cancer (IA2–IIA1), radical hysterectomy with pelvic lymphadenectomy and primary chemoradiation offer comparable cure rates. However, some patients require adjuvant radiation therapy after surgery. Combined modality therapy can lead to significant long-term complications.

Objectives: The objective of this study is to evaluate the incidence of adjuvant radiation therapy after radical hysterectomy and to identify preoperative prognostic factors in early-stage cervical cancer (stages IA2–IIA1) at Lampang Hospital.

Materials and Method: This retrospective cohort study included early-stage cervical cancer patients who underwent radical hysterectomy with pelvic lymphadenectomy (2014–2023). Preoperative risk factors for adjuvant radiotherapy, including tumor size, diagnostic method, tumor type, vaginal bleeding, and suspected vaginal invasion, were analyzed using univariate and multivariate logistic regression in Stata 18.0.

Results: Out of 173 patients, 69 (39.9%) received postoperative adjuvant radiotherapy. Preoperative vaginal bleeding (aOR 2.48, 95% CI: 1.21-5.06; p = 0.013) and suspected vaginal invasion (aOR 10.92, 95% CI: 1.23-97.09; p = 0.032) were significantly associated with the need for adjuvant radiation therapy after radical hysterectomy. Tumor size > 4 cm was associated with an increased risk in the univariable analysis (OR 2.76, p = 0.016). Other factors, including non-SCCA histology and gross lesions, showed trends toward increased risk but did not reach statistical significance.

Conclusion: At Lampang Hospital, 39.9% of patients received postoperative radiation therapy after radical hysterectomy. Preoperative clinical examinations alone are insufficient to predict radiation therapy needs. Advanced imaging techniques, such as CT or MRI,

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GMSMJ 2025; 5 (3): 121-129

are used to aid in treatment planning. In resource-limited settings, evaluations should focus on high-risk patients with vaginal bleeding, suspected vaginal invasion, or tumors > 4 cm.

Keywords: Uterine Cervical Neoplasms, Radical Hysterectomy, Radiotherapy, Adjuvant

Introduction

For early-stage cervical cancer (stages IA2 to IIA1), the primary treatment options are radical hysterectomy with pelvic lymphadenectomy or primary chemoradiation therapy, both of which have comparable cure rates.[1,2] However, after surgery, some patients may require adjuvant radiation therapy to reduce the risk of recurrence,[3] but this can increase the risk of complications from combined modality therapy.[4]

Postoperative adjuvant radiation therapy is used to improve overall and progression-free survival in high-risk patients, such as involvement of lymph nodes, residual tumors, and parametrium invasion.[5] Additionally, it's also given to patients with two or more intermediate-risk factors, like deep stromal invasion, large tumor size, or lymphovascular space invasion (LVSI), based on the 'Sedlis criteria.[3]

Late complications of radical hysterectomy include voiding dysfunction, lymphocyst, and lymphedema.[6] Pelvic radiation therapy, can lead to long-term complications such as vaginal stenosis, dyspareunia, rectal bleeding or stenosis, radiation cystitis, fistulas, and lymphedema.[7] Therefore, receiving combined modality therapy can lead to increased complications from both treatment methods, particularly urethral strictures, radiation cystitis, and vulvovaginal fistulas, which significantly reduce quality of life and may result in further complications.[4,8]

After the FIGO (International Federation of Gynecology and Obstetrics) staging system was revised in 2018[9], imaging, along with clinical examination, became essential for accurately determining the stage of the patient's cancer. The available resources can determine the stage of the patient's cancer using pelvic ultrasound, MRI, CT, or PET/CT scans. These imaging methods give the details about tumor size, parametrial involvement, and lymph node status.[10] In our setting, although CT and MRI are available, prolonged wait times—often exceeding four weeks—limit their routine use. As a result, early-stage cervical cancer is often evaluated based on clinical staging alone. Understanding preoperative prognostic factors for adjuvant radiation therapy is essential for selecting the most appropriate treatment for each patient.

The objective of this study is to evaluate the incidence of adjuvant radiation therapy after radical hysterectomy and to identify preoperative prognostic factors in early-stage cervical cancer (stages IA2–IIA1) at Lampang Hospital, where preoperative access to CT or MRI is limited.

Materials and Method

This retrospective observational cohort study reviewed medical records from the Department of Obstetrics and Gynecology, Lampang Hospital, with approval from the Research Ethics Committee (REC No. 032/66). The inclusion criteria were patients with FIGO stage IA2-IIA1 cervical cancer (classified according to FIGO 2018 but staged without CT/MRI)) who underwent radical hysterectomy with pelvic lymphadenectomy between January 1, 2014, and December 31, 2023.

The primary outcome was the proportion of patients who required adjuvant radiation therapy following

radical hysterectomy. The secondary outcome was the identification of preoperative clinical factors associated with the need for adjuvant radiation therapy, including tumor size, histology, vaginal bleeding, and suspected vaginal invasion. These preoperative risks were based on prognostic factors described in the FIGO 2018 staging system and prior studies on risk stratification (Sedlis criteria and Peters criteria). These included the diagnostic method (Loop Electrosurgical Excision Procedure (LEEP) or gross biopsy), tumor size, cancer type (squamous cell carcinoma (SCCA) or non-SCCA), suspected vaginal extension, lymphovascular invasion, and FIGO stage. CT and MRI findings were excluded from this analysis. Postoperative prognostic factors were identified based on the Peters criteria[5] (at least one of: positive lymph node metastasis, parametrial invasion, or positive surgical margins) and the Sedlis criteria[3] (at least two of: lymphovascular space invasion (LVSI), deep stromal invasion (≥1/3 stromal thickness), or a tumor diameter ≥ 4 cm). General demographic and clinical data collected included age, menopausal status, and presenting symptoms.

The exclusion criteria included patients who received incomplete treatment, those who refused treatment, those with incomplete medical record information, and those who received neoadjuvant chemotherapy.

Patients were divided into two groups. Patients who underwent surgery without additional treatment were classified as the "Surgery alone" group, while those who received adjuvant radiotherapy (including both radiation therapy alone and concurrent chemo-radiation therapy) were classified as the "Surgery and RT" group.

The analysis was performed using Stata software, version 18.0. Descriptive statistics were used to assess the prevalence of adjuvant radiation therapy. Demographic data were presented as percentages, means, standard deviations, medians, and interquartile ranges. Independent t-tests or Mann-Whitney U tests were used to compare continuous variables between groups, and Fisher's exact tests were applied for categorical variables. Statistical significance was set at $p < 0.05$. Univariable and multivariable logistic regression analyses evaluated preoperative prognostic factors for adjuvant radiation therapy, presenting odds ratios (OR), adjusted odds ratios (aORs), and 95% confidence intervals (95% CIs).

Results

In the past 10 years, a total of 202 patients underwent radical hysterectomy with pelvic lymphadenectomy. Out of these, we excluded 29 patients: 18 had other types of cancer, 4 had noncancerous conditions, and 7 had no available information. As a result, 173 patients diagnosed with cervical cancer at stages IA2 to IIA1 underwent surgery. Following surgery, 69 patients (39.9%) received adjuvant radiation therapy.

Table 1 summarizes the baseline characteristics of the study population. The mean age was 48.5 years (range: 24–72 years). Menopause was present in 42.2% of patients, and 39.3% had a history of LEEP. Most patients were classified as FIGO stages IB1–IB3 (85.6%). Histopathological findings showed squamous cell carcinoma (SCCA) in 62.4%, and 84.4% of tumors measured less than 4 cm in diameter. The median tumor size was significantly larger in the Surgery and RT group (2.0 cm vs 1.3 cm, $p = 0.001$), with preoperative tumor size also being significantly greater in this group ($p = 0.007$). Additionally, vaginal bleeding ($p = 0.002$) and suspected vaginal invasion ($p = 0.005$) were more frequent in the Surgery and RT group. However, there were no significant differences between groups in mean age, BMI, menopausal status,

parity, or histopathology. The recurrence rate was 5.77% for the Surgery group and 15.9% for the Surgery plus RT group (p = 0.052). However, this difference was not statistically significant.

Table 1 Baseline characteristics (N=173)

Baseline characteristics	**Total n (%) 173**	**Surgery alone n (%) 104 (60.12)**	**Surgery and RT n (%) 69 (39.88)**	**P-value**
Age (Mean ± SD)	48.5 ± 9.5	47.7 ± 8.7	49.8 ± 10.5	0.137
BMI (Mean ± SD)	23.6 ± 3.5	23.7 ± 3.1	23.5 ± 4.0	0.620
Menopause status				0.365
No	100 (57.8)	63 (60.6)	37 (53.6)	
Yes	73 (42.2)	41(39.4)	32 (46.4)	
Parity				0.459
0	16 (9.2)	11 (10.6)	5 (7.2)	
≥ 1	157 (90.8)	93 (89.4)	64 (92.8)	
Presenting symptoms*				
None	79 (45.7)	57 (71.0)	22 (29.0)	**0.007**
Vaginal bleeding	85 (49.1)	41 (39.4)	44 (63.8)	**0.002**
Abnormal pain	24 (13.9)	17 (16.4)	7 (10.1)	0.248
Diagnostic method				0.052
LEEP or cone biopsy	68 (39.3)	47 (45.2)	21 (30.4)	
Gross lesion	105 (60.7)	57 (54.8)	48 (69.6)	
Tumor size (Pre-op, cm)				**0.007**
≤ 2 cm	94 (54.3)	66 (63.5)	28 (40.6)	
2 – 4 cm.	52 (30.1)	27 (25.9)	25 (36.2)	
> 4 cm.	27 (15.6)	11(10.6)	16 (23.2)	
Median [IQR]	1.5 [0.9-3.0]	1.2 [0.7-3.0]	2.0 [1.2-3.5]	**0.001**
Vaginal Invasion (Suspected)	8 (4.6)	1 (1.0)	7 (10.1)	**0.005**
Pathology				0.506
SCCA	108 (62.4)	67 (64.4)	41 (59.4)	
Non-SCCA	65 (37.6)	37 (35.6)	28 (40.6)	
LVSI positive (Pre-op) (Missing data = 61)**				
	29 (16.8)	15 (14.4)	14 (20.3)	0.596

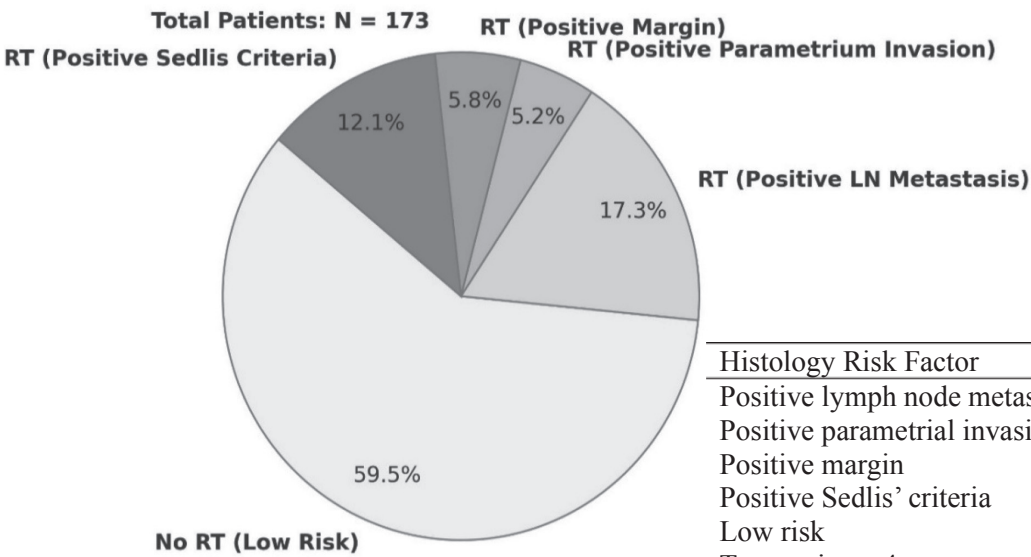
Table 1 Baseline characteristics (N=173) (con.)

Baseline characteristics	Total n (%) 173	Surgery alone n (%) 104 (60.12)	Surgery and RT n (%) 69 (39.88)	P-value
FIGO Stage (Pre-op, 2018)				**0.001**
IA1-IA2	16 (9.2)	15 (14.4)	1 (1.4)	
IB1-IB3	148 (85.6)	88 (84.6)	60 (87.0)	
IIA1-IIA2	9 (5.2)	1 (1.0)	8 (11.6)	
Recurrent rate	17 (9.8)	6 (5.8)	11 (15.9)	0.052

Abbreviations: BMI = Body mass index, LEEP = Loop electrosurgical excision procedure, SCCA = Squamous cell carcinoma, LVSI = Lymphovascular space invasion Pre-op = Pre-operative

Note: * Some patients may have had more than one symptom.

** Missing data = 61 for pre-operative LVSI due to its absence in pre-operative pathology.



Histology Risk Factor	N (%)
Positive lymph node metastasis	30 (17.3)
Positive parametrial invasion	9 (5.2)
Positive margin	10 (5.8)
Positive Sedlis' criteria	21 (12.1)
Low risk	103 (59.5)
Tumor size > 4 cm.	19 (11.0)

Figure 1 Post-operative pathological risk factor of patients (N = 173)

Figure 1 presents the postoperative histology findings used to post-operative pathological risk factor of patients. The most common indications for adjuvant radiation therapy were the presence of positive lymph node metastasis (17.3%) and positive Sedlis criteria (12.1%).

Table 2 Preoperative risk factor for adjuvant radiation therapy after radical hysterectomy in early stage cervical cancer (N = 173)

Risk factor (Pre-operative)	Surgery alone N (%)	Surgery and RT N (%)	Univariable OR (95%CI)	p-value	Multivariable aORs (95%CI)	p-value
Tumor size > 4 cm (n=28)	11 (39.3)	17 (60.7)	2.76 (1.20-6.34)	0.016	2.04 (0.80-5.17)	0.131
Non-SCCA (n=65)	37 (56.9)	28 (43.1)	1.23 (0.66-2.31)	0.506	1.14 (0.57-2.29)	0.708
Gross lesion (n=105)	57 (54.3)	48 (45.7)	1.88 (0.99-3.58)	0.053	0.94 (0.42-2.10)	0.879
Vaginal bleeding (n=85)	41 (48.2)	44 (51.8)	2.70 (1.44-5.07)	0.002	2.48 (1.21-5.06)	0.013
Suspected vaginal invasion (n=8)	1 (12.5)	7 (87.5)	11.63 (1.40-96.77)	0.023	10.92 (1.23-97.09)	0.032

Table 2 identifies preoperative risk factors for adjuvant radiation therapy after radical hysterectomy in early-stage cervical cancer. Vaginal bleeding and suspected vaginal invasion were significantly associated with the need for adjuvant radiation therapy.

In the univariable analysis, vaginal bleeding had an odds ratio (OR) of 2.70 (95% CI: 1.44-5.07; p = 0.002), while in the multivariable analysis, the adjusted odds ratio (aOR) was 2.48 (95% CI: 1.21-5.06; p = 0.013). Similarly, suspected vaginal invasion showed a strong association with adjuvant radiation therapy, with a univariable OR of 11.63 (95% CI: 1.40-96.77; p = 0.023) and a multivariable aOR of 10.92 (95% CI: 1.23-97.09; p = 0.032).

Tumor size > 4 cm was associated with an increased risk in the univariable analysis (OR 2.76; 95% CI: 1.20-6.34; p = 0.016), but this association was not statistically significant in the multivariable analysis (aOR 2.04; 95% CI: 0.80-5.17; p = 0.131).

Other factors, such as non-SCCA histology and the presence of gross lesions, indicated slightly elevated risks but did not achieve statistical significance in either analysis.

Discussion

The study found that 39.9% of patients with cervical cancer stages IA2 to IIA1 received adjuvant radiation therapy post-surgery. This percentage is comparable to other studies conducted between 1995 and 2022 that found figures between 39.5 and 39.7%.[11-13]

In this study, the primary reasons for postoperative radiation therapy were positive lymph node metastasis (17.3%) and positive Sedlis criteria (12.1%). At Lampang Hospital, preoperative evaluation was previously based on clinical staging through physical exams, without the use of CT or MRI scans. With the introduction of FIGO 2018, which incorporates imaging into staging recommendations, the use of preoperative CT or MRI has improved the accuracy of staging and facilitated better identification of stage IIICr (imaging) disease. This, in turn, reduces the likelihood of unnecessary surgery and decreases the proportion of patients requiring postoperative radiation therapy. In resource-limited areas, CT or MRI access is limited to reduce costs, so only certain patients receive scans.

In the early-stage cervical cancer usually doesn't have symptoms until it begins to spread, making it hard to detect.

Pretorius et al. determined that presentation with an abnormal Pap smear is associated with early stage and smaller tumor size, whereas presenting with symptoms other than abnormal Pap smear, such as abnormal vaginal bleeding or pain, is associated with a higher stage but not with disease-free survival.[14] Our study examines indirect factors that influence the need for adjuvant therapy after surgery, based on clinical evaluation and preoperative findings associated with higher disease spread. In our study, identified vaginal bleeding and suspected vaginal invasion were associated with the need for adjuvant radiation therapy.

In our study, preoperative physical examination identified 28 patients with a tumor size of 4 cm or larger. Only 19 individuals had tumors larger than 4 cm, as confirmed by postoperative pathology results. This suggests that the preoperative assessment of tumor size may not have been accurate enough. Pan et al. studied preoperative tumor size assessment compared to final pathology in stage IB2. Physical exams and MRI overestimated tumor size. MRI had 83.2% concordance with final pathology (2-4 cm). Physical exams showed 91.1% concordance with MRI and 80.6% concordance with final pathology.[15]

In cases where the tumor size exceeds 4 cm, it is classified as "bulky IB and IIA cervical cancer" (FIGO 2018 stage IB3 and IIA2). In the United States, where MRI is commonly used for preoperative evaluation, the NCCN (National Comprehensive Cancer Network) guidelines[16] typically recommend definitive chemoradiation over radical surgery with or without adjuvant radiation therapy. In our study, postoperative radiation therapy was administered to 17 patients (60.7%) in this group, which was significant in univariable analysis. Additionally, suspected vaginal invasion (aOR 10.92, 95% CI: 1.23–97.09) and vaginal bleeding (aOR 2.48, 95% CI: 1.21-5.06) were strongly associated with the need for adjuvant radiation therapy. These findings suggest a greater disease burden not fully detected by clinical staging. Therefore, in patients with bulky tumors, vaginal bleeding, or suspected vaginal extension, primary chemoradiation should be considered to avoid unnecessary dual-modality treatment.

Based on our findings, in areas with limited access to CT or MRI, primary radical hysterectomy may be appropriate for patients whose tumors are assessed as less than 4 cm on physical examination. However, patients should be informed that they may need adjuvant radiation therapy if other risk factors are present, like vaginal bleeding or vaginal invasion. For tumors larger than 4 cm, further evaluation with MRI or CT is advised to check for pelvic node metastasis before deciding on treatment. If imaging shows no metastasis, patients can choose either surgery or chemoradiation as their primary treatment. They were informed that surgery carries a high chance of requiring adjuvant radiation therapy, but this approach has the advantage of providing pathological staging.

In settings where advanced imaging is not routinely available, these simple clinical indicators may help guide treatment selection and reduce the likelihood of dual-modality therapy. Identifying high-risk patients before surgery allows clinicians to tailor treatment, improve patient counseling, and reduce unnecessary complications from combined therapies.

The strength of this research is that it uses data collected from a single healthcare center. Preoperative assessments and consistent treatment protocols are homogeneous. The study has limitations, including a small sample size for specific characteristics. The lack of LVSI reporting in pre-surgery pathology results (61 cases),

especially in patients with visible tumors, prevented including this important prognostic factor in the analysis. In the future, addressing these restrictions might increase the accuracy of the research.

Conclusion

The research shows that Lampang Hospital has a 39.9% postoperative radiation therapy rate after radical hysterectomy. Radiation therapy requirements cannot be predicted by preoperative clinical examinations alone. For improved treatment planning, advanced imaging like CT or MRI should be used whenever possible. In regions with limited access, evaluations should be prioritized for high-risk patients who present with vaginal bleeding, vaginal invasion, or tumor size greater than 4 cm.

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สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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Special Article GMSMJ Greater Mekong Subregion Medical Journal

Prevalence of Antiphospholipid Antibodies in Patients with Venous Thromboembolism at Chonburi Hospital

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Received 1 March 2025 • Revised 6 April 2025 • Accepted 6 April 2025 • Published online 1 September 2025

Abstract:

Background: Venous thromboembolism (VTE) is a common condition in clinical practice. Antiphospholipid antibodies (APAs) have been investigated for the causes of thrombosis, which affect anticoagulant duration. The prevalence and association between VTE and APAs influenced the decision to investigate.

Objective: To investigate the prevalence and association between venous thromboembolism (VTE) and antiphospholipid antibodies (APAs).

Materials and Method: A retrospective cross-sectional study was conducted to analyze the medical records of patients with VTE at Chonburi Hospital from January 1, 2021, to April 30, 2024. The data collected included age, sex, body mass index (BMI), location of thrombosis, pregnancy status, underlying diseases, episodes of thrombosis, and presence of APAs. The binary logistic regression analysis focused on the prevalence and risk factors associated with APAs.

Results: In a study of 189 patients with VTE, 42 (22.2%) tested positive for APAs. The average age of the patients was 46 years, and 54.8% were female. Multivariate analysis showed that antiphospholipid antibody-positive patients had a significant association with unprovoked thrombosis, with an adjusted odds ratio of 4.01 (p-value 0.03).

Conclusion: The prevalence of APAs in patients with VTE at Chonburi Hospital was 22.2%. The presence of APAs was significantly associated with unprovoked thrombosis. However, further studies are required to better understand this association.

Keywords: Antiphospholipid antibodies, Prevalence, Venous thromboembolism, Risk association

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GMSMJ 2025; 5 (3): 131-136

Introduction

Venous thromboembolism is common in general practice, with the usual sites of thrombosis being pulmonary embolism (PE) and deep vein thrombosis (DVT). Unusual sites include the cerebral venous sinus, splanchnic vein, renal vein, and other site. Thrombophilia, an increased risk of blood clots, is categorized into inherited and acquired types. Identifying the causes of thrombophilia is crucial, as it determines the length of anticoagulant treatment needed.[1]

APAs include lupus anticoagulant, anti-beta2 glycoprotein IgM, IgG and anticardiolipin IgM, IgG. Their association with VTE varies, with common risk factors like systemic lupus erythematosus (SLE) and unusual site thrombosis.[1,2] The prevalence of antiphospholipid antibodies in patients with thrombosis ranges from 14% to 46%.[3,5,9,14] The risk factors associated with antiphospholipid antibodies positive thrombosis in previous studies include unprovoked DVT, recurrent thrombosis, autoimmune diseases, unusual site thrombosis, and extended thrombosis.[4,5,6,16] The Prevalence and association of VTE and APAs guides our decision to investigate patients, allowing us to focus on those who truly need it and save on cost.

Study design

A retrospective cross-sectional study was conducted to review the medical records of patients with VTE at Chonburi Hospital from 1 January 2021 to 30 April 2024.

Inclusion and Exclusion Criteria

The inclusion criteria comprised patients over 18 years old who were diagnosed with VTE confirmed by imaging. We excluded patients who were superficial thrombosis and inherited thrombophilia. This study was approved by the Research Ethic Committee of Chonburi Hospital.

Materials and method

We collected medical record 189 patients with VTE, using the 10th revision of the International Classification of Diseases (ICD-10) codes I260, I269, I636, I801, I802, I809, I81, I820, I822, I823, I828, I829. The data collected included age, sex, BMI, thrombosis location, pregnancy status, underlying diseases, episodes of thrombosis, and the presence of APAs.

APAs included lupus anticoagulant, Anticardiolipin IgM, IgG and Anti-beta2 glycoprotein IgM, IgG. We gathered data for our investigation by Lupus anticoagulant positivity interpreted according to ISTH guidelines[10] and the timing of the investigation collected before anticoagulant treatment or after withholding anticoagulants for 5-7 days. If reported inconclusive defined to negative.

Anti-beta2 glycoprotein IgM, IgG and Anticardiolipin IgM, IgG defined as positive in a laboratory report, the timing of the investigation collected anytime when diagnosed VTE

Primary and secondary outcome

The primary outcome was the prevalence of APAs in VTE patients at Chonburi Hospital. The secondary outcome was the association of VTE and APAs.

Statistical analysis

Descriptive statistics summarize qualitative variables, like gender, as frequencies or percentages. For quantitative variables, such as age, the mean and standard deviation were used. For comparison of demographic data analysis, use chi-square or Fisher's exact test for categorical data, and apply the independent t-test or Mann-Whitney U test for continuous data as appropriate. Binary logistic regression analysis was performed for related factors in patients with VTE who tested positive

for APAs. P-value < 0.05 was considered statistically significant.

Results

This study included 189 patients with VTE confirmed by imaging. The cohort had a mean age of 48.14 years, with females constituting 67.7% of participants and a mean BMI of 25.23 kg/m^2. Most patients experienced their first thrombotic event. Deep vein thrombosis (DVT) was the most common presentation (42.3%), followed by pulmonary embolism (PE) (33.3%) and cavernous sinus thrombosis (13.2%). Antiphospholipid antibodies (APAs) were detected in 42 patients, representing a prevalence of 22.2% among the study population.

Table 1 Patients characteristics

characteristic	All n = 189	APAs positive n = 42	APAs negative n = 147	*p*-value
Age, years, mean ± SD	48.2 ± 16.5	46.4 ± 16.8	48.6 ± 6.4	0.75[a]
Sex, n (%)				
Female	128 (67.7)	23 (54.8)	105 (71.4)	0.04[b]
BMI (Kg/m^2), mean ± SD	25.3 ± 5.6	24.1 ± 4.5	25.6 ± 5.9	0.23[a]
Pregnancy, n (%)	4 (2.1)	3 (7.1)	1 (0.7)	0.03[c]
Location of thrombosis, n (%)				
Pulmonary embolism	63 (33.3)	10 (23.8)	53 (36.1)	0.14[b]
Deep vein thrombosis	80 (42.3)	19 (45.2)	61 (41.5)	0.67[b]
Splanchnic vein thrombosis	15 (7.9)	4 (9.5)	11 (7.5)	0.75[c]
Cavernous sinus thrombosis	25 (13.2)	7 (16.5)	18 (12.2)	0.46[b]
Renal vein thrombosis	1 (0.5)	0 (0)	1 (0.7)	1.00[c]
Others	5 (2.6)	2 (4.8)	2 (1.4)	0.18[b]
Underlying disease, n (%)				
SLE	17 (7)	6 (14.3)	11 (7.5)	0.22[c]
Cancer	18 (9.5)	5 (11.9)	13 (8.8)	0.56[c]
Recurrent thrombosis, n (%)	29 (15.3)	7 (16.7)	22 (15)	0.79[b]
Unprovoked thrombosis, n (%)	146 (77.2)	39 (92.9)	107 (72.8)	0.01[b]

Data was presented as n (%), mean (SD). A *p*-value < 0.05 indicated statistically significant; [a]Unpaired t-test (equal variate was assumed); [b]chi-square test; [c]Fisher's exact test; *analyse without missing data.
Abbreviation: APAs, antiphospholipid antibodies; SD, standard deviation; BMI, body mass index; SLE, systemic lupus erythematosus

Univariate analysis of the association between APAs-positive and VTE patients indicated that the factors associated with positive APAs included female sex [OR = 2.06 (95% CI 0.45, 2.88), $p = 0.04$] and unprovoked thrombosis [OR = 4.86 (95% CI 0.72, 3.31), $p = 0.01$]. When the two factors were analyzed using multivariate analysis, only unprovoked thrombosis [adjusted OR = 4.01 (95% CI 1.16, 14.16), p-value = 0.03] demonstrated a significant association with the detection of these antibodies, whereas female did not exhibit statistical significance.

Table 2 The related factor of a positive APAs in VTE patients at Chonburi Hospital

Factor	Univariable			Multivariable		
	OR	95%CI	p-value	OR*	95%CI	*p*-value**
SLE	2.06	0.71-5.9	0.18			
Unprovoked thrombosis	4.86	1.42-16.6	0.01	4.01	1.16-14.16	0.03
Unusual site	1.55	0.72-3.31	0.26			
Recurrent thrombosis	1.14	0.45-2.88	0.79			
Female	2.06	1.02-4.18	0.04	2.08	0.95-4.52	0.06
Age < 50 years old	1.38	0.68-2.82	0.36			

OR*, an adjusted odds ratio

p-value < 0.05 indicated statistical significance by Binary Logistic regression

Abbreviation: OR, Odds ratio; CI, Confident interval; SLE, systemic lupus erythematosus

Discussion

The prevalence of APAs (22.2%) in our cohort of patients with VTE at Chonburi Hospital fell within the range reported in previous studies[3,5,9] that APAs were positive in venous thromboembolism patients at 14%, 15%, and 21.7%, respectively

Our study identified that unprovoked thrombosis increased the likelihood of detecting APAs in patients with VTE by fourfold (p-value < 0.05). This finding is consistent with previous study, which recognized unprovoked DVT and recurrent DVT as risk factors. However, it is noteworthy that research was limited to deep vein thrombosis and did not include other thrombosis location.[4] A study reported a 14% positivity rate for APAs in cases of unprovoked thrombosis[7], which aligns with our findings.

Previous studies have identified systemic lupus erythematosus (SLE), female gender, recurrent thrombosis, and thrombosis at unusual sites as risk factors for APAs positivity.[4-6,16] However our study did not find these factors to be significant. We observed that thrombosis in cancer patients was not associated with APAs. Conversely, previous study reported a 60.6% prevalence of APAs linked to recurrent thrombosis.[6]

The limitations of this study include its retrospective design, incomplete data, and missing laboratory investigations, which may have affected the accuracy of the results. Future research should involve a prospective cohort study to ensure comprehensive data collection, potentially incorporating additional factors, such as drug use and other underlying diseases, to better understand the association of factors.

Conclusion

The prevalence of APAs in patients with VTE at Chonburi Hospital was 22.2%. The presence of APAs was significantly associated with unprovoked thrombosis [adjusted OR = 4.01 (95% CI 1.16, 14.16), *p*-value = 0.03]. However, further studies are needed to better understand this association.

Acknowledgement

Special thanks to Dr. Sittikorn Srivorrapatarakul for his recommendation on the statistical analysis.

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A Study of Behavior of Applying Knowledge of Learners from the Course SIID 529 Effective Clinical Teaching of Faculty of Medicine Siriraj Hospital, Mahidol University

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Received 1 April 2025 • Revised 12 June 2025 • Accepted 20 June 2025 • Published online 1 September 2025

Abstract:

Background: SIID 529 is a course at Siriraj Hospital, Mahidol University, designed to enhance clinical teaching skills among medical residents. This study evaluates how learners apply teaching knowledge in practice through self- and peer-assessments. The results aim to inform future instructional strategies and educational policies.

Objective: To assess and compare learners' knowledge application behavior following participation in SIID 529 using self-assessment and peer-assessment tools.

Materials and Method: The study involved 840 participants, including medical residents, fellows, and their colleagues. Two validated questionnaires assessed knowledge application across four domains: clinical supervision, ward rounds, giving feedback, and teaching on the run. A total of 268 self-assessments and 240 peer assessments were collected (response rates: 31.40% and 28.54%). Data were analyzed using descriptive and non-parametric comparative statistics.

Results: Overall knowledge application was rated at a high level (Self-assessment: $\overline{X} = 3.73$; Peer-assessment: $\overline{X} = 3.77$). Statistically significant differences ($p < 0.05$) were observed based on academic year, study status, academic performance, and learning environment. The highest application levels were found among graduates and fellows, especially those from the academic year 2021, while current residents in 2023 reported lower application. Learners with an A+ grade and those in supportive environments showed significantly higher behavior scores.

Conclusion: The SIID 529 course has a substantial impact on enhancing physicians' teaching behaviors. Institutions should support knowledge application by providing structured training opportunities, reducing teaching barriers, and fostering environments that encourage clinical education. These findings support the importance of faculty development for medical educators and suggest areas for future improvement in training programs.

Keywords: Clinical teaching, Learner, Knowledge application, Resident

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GMSMJ 2025; 5 (3): 137-148

Introduction

The course SIID 529: Effective Clinical Teaching is an elective course offered as part of the Graduate Diploma in Clinical Medical Sciences, which has been continuously conducted since 2019 at the Faculty of Medicine Siriraj Hospital, Mahidol University. The course content comprises two components: 1) Theoretical Component; Learner's study online teaching materials from the "Essential Skills for Clinical Teachers" course via the website shee.si.mahidol.ac.th prior to classroom sessions. 2) Practical Component; Classroom-based learning and practice sessions, where learners are divided into small groups to engage in skill-based activities covering: 1) Clinical supervision, 2) Ward rounds, 3) Giving feedback, and 4) Teaching on the run. As many medical residents play significant roles in teaching medical learners and junior residents, they are responsible for transmitting core principles of clinical teaching. Therefore, it is essential that they possess appropriate knowledge and teaching skills, such as questioning techniques, feedback delivery, clinical supervision, time-constrained teaching, interactive teaching, small-group teaching, bedside teaching, ward round instruction, clinical performance assessment, and attitude teaching. Enhancing clinical teaching skills is thus a crucial and beneficial endeavor that can be applied in mentoring junior learners effectively.

Essential clinical teaching competencies such as bedside teaching, effective feedback, clinical supervision, and time constrained "teaching on the run" have been well documented in both Thai and international literature. Bedside teaching, for instance, improves diagnostic skills and is valued by learners and patients alike.[1] A systematic review highlighted that quality feedback, teaching planning, and intrinsic educator traits significantly enhance student learning and patient care.[2] Furthermore, structured "Residents as Teachers" programs in surgical and family medicine residencies foster teaching confidence and long term involvement in education.[3,4] The book Essential Skills for a Medical Teacher emphasizes these domains as foundational for clinical educators.[5] Such global and local evidence supports the need for SIID 529 to systematically equip residents and fellows at Siriraj Hospital with formal clinical teaching skills.

Behavioral studies can be conducted in two ways: 1) Direct observation, and 2) Indirect methods, such as interviews, surveys, or experimental studies. Using questionnaires is considered an appropriate method for studying the behaviors of a large population, including those in remote areas. Moreover, participants can report concealed or sensitive behavioral data.[6] Additionally, Donald L. Kirkpatrick proposed a four-level model for evaluating training programs: 1) Reaction – participants' satisfaction and impressions toward the program 2) Learning – assessment of knowledge gained 3) Behavior – the extent to which behaviors have changed and knowledge is applied in practice 4) Results – impact of the training on the organization.[7] In the SIID 529 course, evaluation at the reaction level is conducted through a satisfaction survey, while learning is assessed through examinations and grading. However, there has not yet been an evaluation at the behavior level to determine whether learners apply the acquired knowledge and skills in their clinical practice. Therefore, this study employs an indirect method of behavioral assessment through questionnaires completed by both learners and their colleagues.

Given the importance of clinical teaching and behavioral evaluation, the researcher is interested in studying the application of knowledge by learners from the SIID 529: Effective Clinical Teaching course at the Faculty of Medicine Siriraj Hospital, Mahidol University. The findings from this study will contribute to effective outcome monitoring, provide evidence-based information for educators to improve instructional activities, and offer valuable insights for administrators in formulating future directions and policies.

Materials and method

Ethical Consideration : This study was approved by the Institutional Review Board (IRB) with the Certificate of Approval (COA) No. Si 527/2024.

1. Research design

Subject : The study population consisted of 1,510 medical residents and clinical fellows who had enrolled in the course SIID 529: Effective Clinical Teaching, Faculty of Medicine Siriraj Hospital, Mahidol University, during the academic years 2019 to 2023.

Sample : A total of 840 medical residents and clinical fellows who had previously completed the SIID 529 course were invited to participate in the study, along with 840 colleagues for peer assessments.

The required sample size was determined using a rule of thumb recommended by Hair et al., suggesting a minimum of 20 participants per observed variable.[8] With four behavioral indicators, the minimum sample was set at 80. To ensure sufficient data for subgroup comparisons using non-parametric tests (Mann–Whitney U and Kruskal–Wallis), and to account for potentially low response rates typical in survey research, the sample size was increased tenfold. This decision was based on Becker and Huselid's findings indicating an average response rate of 6.28% in similar behavioral studies.[9] Consequently, 840 participants were invited to complete the self-assessment and peer-assessment questionnaires.

The minimum acceptable number of participants per group was calculated by proportionally distributing the total sample across academic years and departmental affiliations, following the method suggested.[10] The simple random sampling method was used to select participants within each group, and data collection was concluded once the target number of responses was achieved.

Table 1 Number of Population and Sample Classified by Academic Year

Academic Year	Population (persons)			Sample (persons)		
	Pre-clinical	Clinical	Total	Pre-clinical	Clinical	Total
2019	8	276	284	4	154	158
2020	9	296	305	5	165	170
2021	9	289	298	5	161	166
2022	9	301	310	5	167	172
2023	6	307	313	3	171	174
Total	**41**	**1,469**	**1,510**	**23**	**817**	**840**

Independent variable - General Information of Learners

1. **Academic Year:** 1) 2019, 2) 2020, 3) 2021, 4) 2022, 5) 2023
2. **Department:** 1) Clinical, 2) Pre-clinical
3. **Sponsoring Organization:** 1) Faculty of Medicine Siriraj Hospital, 2) Other hospitals / medical schools, 3) Free train
4) **Study Status:** 1) Studying in the residency program, 2) Studying in the fellowship program,
3) **Graduated**
5) **Academic Performance:** 1) A+, 2) A, 3) B+, 4) B, 5) Cannot recall
6) **The environment that facilitates knowledge application includes:**
1) Facilitates application 2) Does not facilitate application 3) Not sure

↓

Intervention - Course SIID 529 Effective Clinical Teaching

This study focuses on exploring the behaviors of learners in applying knowledge acquired from the course SIID 529: Effective Clinical Teaching, offered by the Faculty of Medicine, Siriraj Hospital, Mahidol University. The course emphasizes the development of teaching skills in a clinical setting through the following key areas: 1) Clinical Supervision 2) Ward Round 3) Giving Feedback 4) Teaching on the Run

↓

Dependence variable - Learners' Knowledge Application Behaviors

1) **Clinical Supervision:** Learners demonstrate the application of supervision processes through structured teaching approaches, including; Briefing, Clinical practice and Debriefing.
2) **Ward Round:** Learners exhibit behaviors that reflect the application of bedside teaching principles before, during, and after the ward round, including; Deriving teaching points from patient-related data, Ensuring patient comfort and maintaining respect for patients throughout the teaching process, and Creating opportunities for providing feedback to medical learners during or after the round.
3) **Giving Feedback:** Learners apply feedback principles and techniques in clinical teaching, such as; Building a trusting environment and setting shared goals, Providing feedback based on direct observation, Delivering feedback at appropriate times and with consistency, Start with a self-assessment by learners, followed by positive feedback, Give specific negative feedback with a focus on observable behaviors, Provide feedback in an appropriate amount, focusing on key issues that are important and actionable, Offering guidance and jointly planning improvements.
4) **Teaching on the Run:** Learners apply the 1-Minute Teaching, 5-Step Microskills in their clinical teaching practices; 1) Make a commitment, 2) Explore reasons, 3) Teach general rules, 4) Reinforce what was right, 5) Correct mistakes.

Figure 1 Conceptual Framework for the Study

2. Research Instruments

The instrument used in this study is a Questionnaire for Assessing Learners' Knowledge Application Behaviors from the course SIID 529: Effective Clinical Teaching. There are two versions of the questionnaire; 1) Self-assessment Questionnaire 2) Peer-assessment Questionnaire. Each questionnaire consists of three parts:

Part 1: General Information of Respondents

This section includes demographic and background information of the respondents, presented in a checklist format.

Part 2: Knowledge Application Behaviors of Learners

This section evaluates the behaviors related to knowledge application derived from the course content, categorized into four domains; 1) Clinical supervision, 9 items 2) Ward round, 14 items 3) Giving feedback, 10 items and 4) Teaching on the run, 6 items. All items in this section are measured using a 5-point Likert rating scale (interval scale). The scoring criteria for assessing learners' application of knowledge are as follows:

Always used (100% performance), 5 Score.

Almost always used (67–99% performance), 4 Score.

Sometimes used (34–66% performance), 3 Score.

Rarely used (1–33% performance), 2 Score.

Never used (0% performance), 1 Score.

The interpretation of mean scores is based on the class interval width calculation[11] as follows:

1.00 – 1.50 = Very Low Level of Knowledge Application Behavior.

1.51 – 2.50 = Low Level of Knowledge Application Behavior.

2.51 – 3.50 = Moderate Level of Knowledge Application Behavior.

3.51 – 4.50 = High Level of Knowledge Application Behavior.

4.51 – 5.00 = Very High Level of Knowledge Application Behavior.

Part 3: Open-ended Suggestions

This section consists of open-ended questions, allowing respondents to provide comments and additional suggestions freely.

3. Data collection & Quality control

1) A review of relevant literature and textbooks was conducted to explore teaching techniques including Clinical Supervision, Ward Round, Giving Feedback, and Teaching on the Run, as well as previous research studies related to knowledge application behaviors.

2) Study the guidelines and methods for creating questionnaires from textbooks, documents, and research papers, then develop questionnaire. Afterward, have your advisor review the questionnaire.

3) Verify the content validity with three experts, who are specialists in the subject and instructors of the course, including: 1) Rater 1 (clinical education expert), 2) Rater 2 (clinical education expert), and 3) Rater 3 (clinical education expert). Calculate the Item-Objective Congruence (IOC) index for each question using the formula, with an IOC value greater than 0.5 considered acceptable for use. (IOC for the Self-assessment Questionnaire = 0.957 and the Peer-assessment Questionnaire = 0.930).

4) Test the Reliability by administering the revised questionnaire as a tryout with 30 participants who had previously attended the "Essential Skills for Clinical Teachers" training but are not part of the target population. The results will be analyzed to calculate the reliability of the instrument using Cronbach's Alpha Coefficient. This method assesses the

reliability of a research instrument by collecting data from the tryout group once, and a coefficient of 0.8 or higher is considered acceptable[12], as shown in Table 2.

Table 2 Reliability of the Questionnaires

Topic	Questionnaire 1: Self-assessment (Learners)	Questionnaire 2: Peer assessment (Colleagues)
1) Clinical supervision	0.905	0.894
2) Ward round	0.911	0.927
3) Giving feedback	0.950	0.912
4) Teaching on the run	0.934	0.907
Total	**0.935**	**0.972**

5) Data collection was conducted through two formats: paper-based distribution and online questionnaires, as detailed below:

5.1) Paper-Based Distribution: The researcher prepared questionnaire packages with two parts: (1) Self-assessment by the learner and (2) Peer assessment by a colleague, both marked with matching codes to indicate academic year and department. Each package included a return envelope and an instruction sheet emphasizing voluntary participation, submission deadlines, and the choice between paper-based and online formats to prevent duplicate responses. Formal request letters were sent to institutions to assist with distribution and collection.

5.2) Online Format: Emails were sent to participants with academic-year-specific links to the online questionnaires. Participants were instructed to complete the self-assessment first and then forward the peer assessment link to a colleague. The peer respondent was requested to submit the form within three weeks. Participation remained voluntary, with a reminder to choose only one submission method (paper or online) to avoid duplication.

6) Collect and verify the completeness of the questionnaire.

7) The researcher conducts the analysis of the questionnaire.

Statistical analysis

1. Descriptive Statistics:

1) Frequency and percentage were used to analyze the general demographic data of the respondents.

2) Mean and standard deviation were used to analyze the levels of learners' knowledge application behavior, based on self-assessment and peer assessment.

2. Inferential Statistics: Comparative analysis was conducted to examine the differences between learners' knowledge application behavior and their demographic data. Preliminary assumption testing revealed that the dependent variables were not normally distributed. Therefore, non-parametric tests were used the Kruskal-Wallis Test was applied when comparing more than two groups and the Mann-Whitney U Test was applied when comparing two groups.

Results

The researcher received 268 completed questionnaires from learners (Response rate = 31.40%) and 240 completed questionnaires from colleagues of the learners (Response

rate = 28.54%). These response rates were deemed acceptable based on the sample size calculation, and data collection was stopped. Regarding the general information of the learners, the majority were from the 2023 academic year, totaling 130 learners (48.5% of all respondents). For the years 2019-2022, the number ranged from 30 to 42 learners (11.2% – 15.7%). The majority were from the clinical department, totaling 247 learners (92.2%), while 21 learners (7.8%) were from the preclinical department. Regarding their affiliations, 132 learners (49.3%) were affiliated with the Faculty of Medicine Siriraj Hospital, 87 learners (32.5%) were affiliated with other medical schools, and 48 learners (17.9%) were from the Free train program. The majority were enrolled in the residency program, with 231 learners (86.2%), while 25 learners (9.3%) were in advanced programs. Most learners reported that they would not remember their academic results (201 learners, 75.0%) and believed that the environment facilitated the application of knowledge (149 learners, 55.6%). The next largest group was unsure 94 learners (35.1%). Among the colleagues who completed the questionnaires, the majority were residents 172 respondents (72.9%), followed by advanced program physicians 39 respondents (16.5%), and teaching assistants 15 respondents (6.4%). The results of the research are summarized as follows:

1) Knowledge Application Behavior of Learners from the Course SIID 529 Effective Clinical Teaching at Faculty of Medicine Siriraj Hospital, Based on self-assessment and peer assessment.

Table 3 Knowledge Application Behavior of Learners

Item	Self-assessment (n = 268)			Peer assessment (n = 240)		
	$\overline{X}$	S.D.	Behavior level	$\overline{X}$	S.D.	Behavior level
Clinical supervision	3.86	0.49	High	3.86	0.49	High
Ward round	3.63	0.53	High	3.70	0.52	High
Giving feedback	3.72	0.60	High	3.75	0.58	High
Teaching on the run	3.81	0.62	High	3.83	0.59	High
Overall	**3.73**	**0.49**	**High**	**3.77**	**0.50**	**High**

Table 3 shows that the overall knowledge application behavior from the SIID 529 Effective Clinical Teaching course is at a high level. When categorized by topic, all items were rated at a high level. The topics with the highest mean scores, in order, are Clinical supervision, followed by Teaching on the Run, Giving feedback and Ward round respectively.

2) Comparison of learners' behavior in applying knowledge from the SIID 529: Effective Clinical Teaching course, conducted by the Faculty of Medicine Siriraj Hospital, Mahidol University, based on general information of the learners.

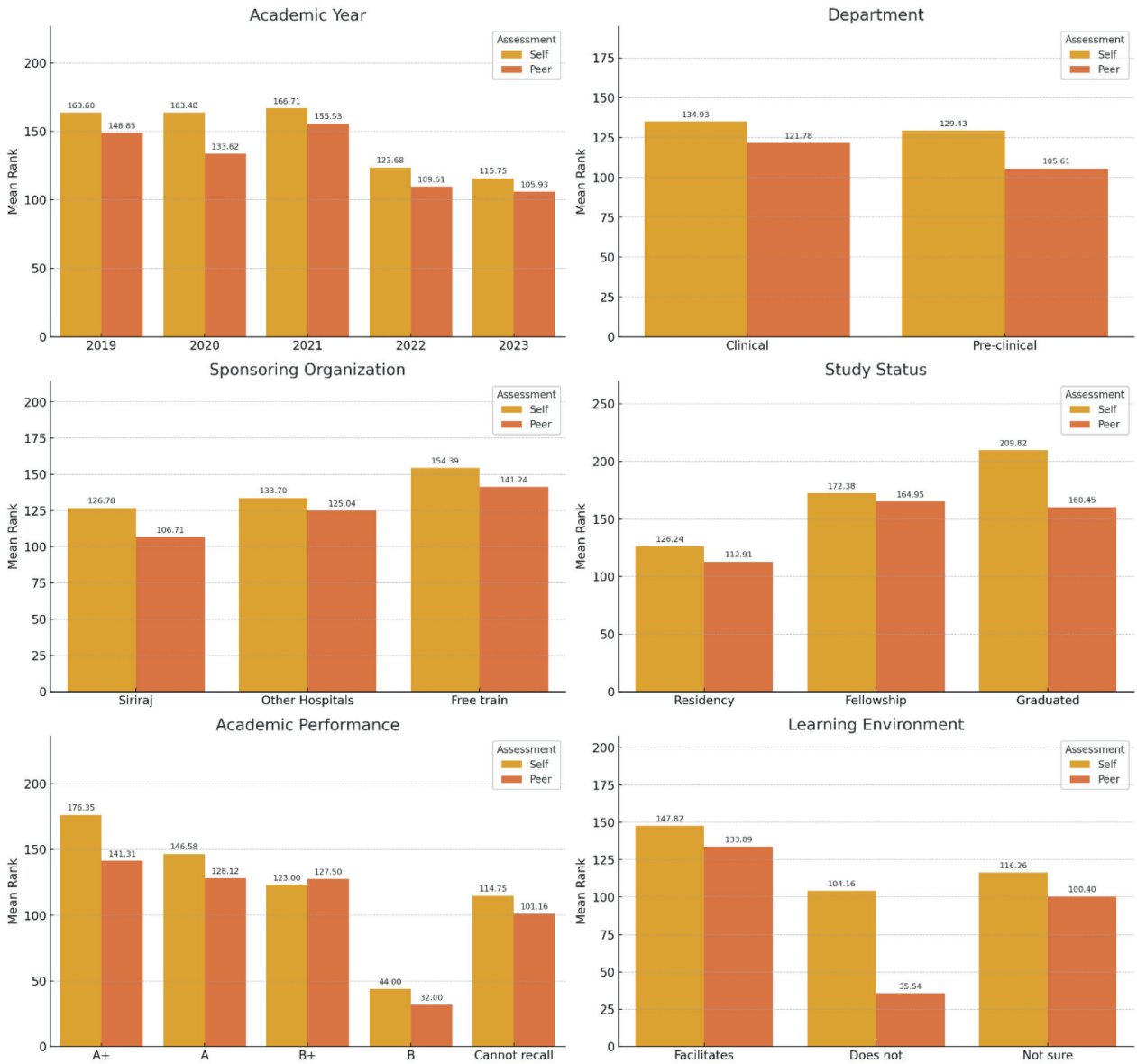

Figure 2 Comparison of learners' behavior in applying knowledge, based on general information of the learners

Figure 2 shows comparison of learners' knowledge application behavior from the SIID 529: Effective Clinical teaching course, based on self-assessment and peer-assessment, across six categories: academic year, department, sponsoring organization, study status, academic performance, and learning environment. Bars represent mean rank scores from non-parametric analyses (Kruskal-Wallis and Mann-Whitney U tests), with numeric values displayed on top.

Statistically significant differences ($p < 0.05$) were observed in comparisons by: Academic year: Learners from 2019–2021 showed significantly higher knowledge application than those from 2023.

Study status: Graduates and fellows reported higher behavioral application than those still in residency.

Academic performance: Learners with an A+ grade applied their knowledge more effectively than lower-performing peers.

Learning environment: Supportive environments correlated with higher application behavior scores in both assessments.

No statistically significant differences were found in:

Department: Clinical and pre-clinical learners showed similar application behaviors, suggesting that departmental affiliation may not be a determining factor.

Sponsoring organization: While peer-assessment indicated significant differences favoring Free-train participants, self-assessment did not reach statistical significance. This discrepancy may reflect differences in external perception or institutional support.

Discussion

Knowledge Application Behavior of Learners from the Course SIID 529 Effective Clinical Teaching at Faculty of Medicine Siriraj Hospital, Based on self-assessment and peer assessment, the overall average scores and all specific aspects met the required criteria. All aspects were rated at the highest level. Additionally, both self-assessments and peer evaluations ranked the highest-scoring aspects in the same order, indicating that the knowledge application behavior in each aspect follows the same direction. When analyzing specific aspects in detail, it was found that learners applied their knowledge most prominently in the Clinical Supervision aspect. They prioritized patient safety over allowing medical students to gain hands-on procedural experience. This is because physicians are instilled with the principle that patient safety is the top priority, this aligns with research findings indicating that the medical profession is one of the occupations with the highest responsibility index score (100%). This demonstrates that, from the past to the present, physicians remain highly conscious of their societal responsibility to provide patient care.[13] As medical students learn while caring for patients, errors may occur, and at times, their clinical decision-making may be less developed compared to faculty members or senior physicians. Therefore, it is essential to anticipate potential errors, minimize them as much as possible, and, when they do occur, ensure a supportive environment that allows medical students to reflect on their actions and learn from their mistakes rather than feeling blamed.[14] Due to time constraints, high patient loads, and heavy workloads, the application of knowledge in the Ward Round aspect was the least prominent. In this setting, focused teaching is employed, selecting specific key topics that are appropriate for the medical students' level and the available time. Meanwhile, the Teaching on the Run aspect ranked second in terms of knowledge application, where, after understanding the underlying reasons, general principles were also taught to medical students. Regarding the Giving Feedback aspect, the most applied behavior was allowing medical students to ask questions while demonstrating active listening. This aligns with the principle that "There is a tradition for doctors to teach their colleagues and medical students," emphasizing the importance of sharing expertise and transferring medical knowledge.[15] However, this has become increasingly challenging due to lack of time, increasing patient loads, insufficient teaching knowledge, and inadequate training. Clinicians need to enhance their knowledge on how to motivate learners and provide constructive feedback.[16]

The results demonstrated meaningful patterns in how learners applied knowledge from the SIID 529 course, which are discussed in detail below across key variables.

Academic Year: Learners from the academic years 2019, 2020, and 2021 exhibited the highest knowledge application scores. This suggests that the effectiveness

of SIID 529 is most evident after learners have progressed beyond the residency phase and have had more time and opportunities to apply their teaching skills in practice. This finding supports the Kirkpatrick Model (Level 3: Behavior), which emphasizes that behavior change requires not only the acquisition of knowledge but also the chance to apply it in real-life contexts over time.[17] Bilal, et al. (2017) similarly noted that faculty development is most impactful when it includes follow-up opportunities for reflection and implementation.[18]

Study Status: Graduates and fellows scored significantly higher than those currently in residency. This may reflect increased professional confidence and greater autonomy in teaching roles. The finding aligns with Adult Learning Theory[19], which posits that adult learners are more motivated to apply knowledge when it is directly relevant to their roles and responsibilities.

Academic Performance: Learners with an A+ academic grade demonstrated significantly greater application of teaching behaviors. This result can be interpreted through Bandura's Self-Efficacy Theory[20], where individuals with stronger self-belief are more likely to apply knowledge assertively and persistently. Additionally, Alam, et al.[21] found that high-performing individuals were more proactive in sharing knowledge and mentoring others-behaviors consistent with those promoted in SIID 529.

Learning Environment: Participants who reported being in a supportive environment had the highest mean scores. These environments likely include reasonable workloads, opportunities to teach students, and collaboration with motivated peers - factors that reinforce the practical use of teaching skills. According to Green and Kreuter's PRECEDE–PROCEED Model[22], behavior is shaped by predisposing, enabling, and reinforcing factors. A learning-friendly environment enables the application of newly acquired skills and reinforces them through organizational support and recognition.

This is further supported by literature on organizational performance, which identifies workplace responsibility, knowledge exchange, and supportive infrastructure as essential for enhancing professional behavior and effectiveness.[23]

Non-Significant Differences: No statistically significant differences were found between learners from clinical and pre-clinical departments, suggesting that departmental affiliation does not influence the application of teaching knowledge. This finding implies that learners, regardless of specialty, can benefit equally from the course. Additionally, although peer-assessment indicated higher scores for Free-train participants, self-assessment did not show a significant difference based on sponsoring organization. This discrepancy may reflect external perceptions shaped by institutional support, teaching culture, or frequency of teaching opportunities.

Study Limitations

1) The use of self- and peer-report instruments may be subject to response bias, such as social desirability or recall limitations.

2) The response rate was modest (approximately 30%), which may limit the generalizability of the findings across all participants in the SIID 529 course.

3) The study could not control for external variables, such as patient load, actual teaching responsibilities, or departmental teaching culture, which may have affected learners' ability to apply knowledge.

Practical Implications

1) For educators and curriculum planners: Structured post-course interventions

such as peer coaching, mentoring, or reflective practice groups may help learners sustain behavioral change.

2) At the organizational level: Institutions should consider strategies to promote teaching-conducive environments by managing clinical workloads, providing feedback systems, and encouraging teaching roles among trainees.

3) The dual-assessment approach (self and peer) enhances the validity of outcome evaluation and should be adopted in similar programs.

Recommendations for Future Research

1) Future studies should incorporate qualitative or mixed methods approaches (e.g., interviews or observations) to deepen the understanding of how and why teaching behaviors are applied.

2) Including perspectives from medical students who receive instruction from SIID 529 graduates may offer indirect evidence of course effectiveness.

3) Expanding the sample size and including a broader range of institutions would improve the external validity of future findings.

Conclusion

The study on learners' knowledge application behavior from the SIID 529: Effective Clinical Teaching course at the Faculty of Medicine Siriraj Hospital demonstrated that the course content is highly beneficial for physicians. As senior physicians are inevitably responsible for teaching and mentoring junior doctors, the course contributes meaningfully to enhancing their teaching competency. Institutions should therefore manage clinical workload and time allocation effectively, while providing formal training in clinical supervision, ward rounds, teaching on the run, and giving feedback to empower their role as educators.

Moreover, fostering a supportive environment - such as providing opportunities to teach, manageable patient loads, and collaborative teaching culture - can enhance the real-world application of teaching skills. These improvements ultimately benefit both medical students and patients.

Although most learners were able to apply their knowledge in real practice, the extent of application varied depending on individual and contextual factors.

Funding

This study was funded by the medical education Fund (Fund 16), Faculty of Medicine Siriraj Hospital, in the year 2024.

Conflict of Interest

The authors have no conflicts of interest.

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Prevalence of Psychiatric Symptoms Associated with Vitamin D Level in Long COVID: Preliminary Study

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Received 3 March 2025 • Revised 30 April 2025 • Accepted 13 May 2025 • Published online 1 September 2025

Abstract:

Background: Long COVID is a condition where individuals continue to experience persistent symptoms after recovering from the initial COVID-19 infection. Vitamin D is one of the vital minerals for maintaining normal health conditions and may be associated with the psychiatric symptoms of long COVID.

Objective: To examine the prevalence of psychiatric symptoms in long COVID-19 patients in association with vitamin D levels.

Materials and Method: This study employed a cross-sectional descriptive design, focusing on 170 patients who had previously contracted the COVID-19 virus at Foresta Clinic. The data collected included demographic data, vitamin D levels, and psychiatric symptoms in long COVID (anxiety, depression, and sleep disorders).

Result: The study results indicated a female-to-male ratio of 1.1:1 among the patients, with a mean age of 45.87 ± 8.65 years. Additionally, 62.4% had received three doses of the COVID-19 vaccine. The median blood vitamin D level was 22.96 ng/mL (IQR 18.77, 31.7), with 41.2% of participants showing insufficiency, 30.6% showing deficiency, and 28.2% having sufficient levels. Overall, psychiatric symptoms were found in 30.0% of the patients, with anxiety occurring in 15.3%, depression in 7.1%, and sleep disorders in 21.2%. Participants with psychiatric symptoms had significantly lower blood vitamin D levels compared to those without symptoms ($p < 0.05$). The prevalence of psychiatric symptoms was highest among those with vitamin D deficiency (46.2%), followed by those with vitamin D insufficiency (25.7%), and it was lowest in the vitamin D sufficient group (18.8%).

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GMSMJ 2025; 5 (3): 149-158

Conclusion: Maintaining and assessing vitamin D levels in long-term COVID patients may help prevent or reduce the severity of psychiatric symptoms. Further research is needed to evaluate the effectiveness of vitamin D supplementation in long COVID patients with low vitamin D levels, as well as to monitor and assess psychological tests continuously.

Keywords: Long COVID, COVID-19, Psychiatric Symptoms, Vitamin D

Introduction

Coronavirus Disease 2019, also known as COVID-19, is caused by a respiratory infection from a newly emerged strain of the coronavirus, SARS-CoV-2, which underwent a natural mutation to form a new variant. The subsequent global outbreaks have caused significant repercussions worldwide, influencing public health, economies, and societies.[1] COVID-19 can lead to both immediate and prolonged health issues. Even after recovery, some patients may not fully regain their pre-illness physical condition and may experience persistent or new symptoms. People commonly refer to this condition as long COVID or post-acute sequelae of SARS-CoV-2 infection (PASC). The World Health Organization (WHO) defines "long COVID" as a condition that occurs in individuals with a probable or confirmed history of SARS-CoV-2 infection. It typically occurs within 3 months of the initial infection and involves symptoms lasting for at least 2 months, which cannot be attributed to other diagnoses.[2] The National Institute for Health and Care Excellence (NICE) defined post-COVID-19 syndrome as "signs and symptoms that develop during or after acute COVID-19 for more than 4 weeks. This condition is divided into 2 phases: post-acute COVID-19, which refers to signs and symptoms that last or recur between 4 and 12 weeks after infection, and chronic COVID-19, which refers to signs and symptoms that persist for 12 weeks or longer." [3]

Long COVID can be found in 10-30% of COVID-19 patients[4], with over 200 symptoms impacting various organ systems.[5] These symptoms include general symptoms, respiratory system, cardiovascular system, nervous system, skin system, gastrointestinal system, musculoskeletal system, as well as psychological symptoms that may also occur in long COVID. A study of 120,970 COVID-19 patients found a prevalence of mental health disorders in 20.3% of patients with long COVID. The study classified these disorders into the following categories: 13.6% had post-traumatic stress disorder (PTSD), 18.9% had anxiety, 16.1% had depression, and 17.8% had sleep disorders.[6] Psychiatric disorders can arise from various factors, including encephalitis/cerebral hypoxia, medical interventions, physical isolation, psychosocial impact, and social stigma. Additionally, increased inflammation in the body caused by SARS-CoV-2 infection (high levels of CRP, IL-6, and ferritin) heightens the risk of psychiatric symptoms like depression.[7] Furthermore, deficiencies in essential vitamins and minerals can also negatively impact overall bodily function.[8] Vitamin D, especially, is stored in fatty tissues and is a fat-soluble vitamin with a chemical derived from cholesterol. It plays a significant role in supporting overall health by regulating the immune system's response—both innate and adaptive immunity.[9] Additionally, it supports the immune responses to antimicrobial and antiviral agents.[10]

There are currently no conclusive findings about the role of vitamin D in residual psychiatric symptoms after COVID-19

recovery. Therefore, we are interested in exploring the relationship between vitamin D levels and the prevalence of psychiatric symptoms associated with long COVID. The findings from this study will enhance our understanding of the role of vitamin D deficiency or insufficiency and its potential link to psychiatric symptoms in long COVID patients.

Materials and Method

This study was a descriptive cross-sectional study, approved by the Ethics Committee of Mae Fah Luang University (COA: 166/2024, EC 24104-20). The sample group consisted of 170 patients who were previously infected with COVID-19, confirmed by either the Covid-Ag or RT-PCR methods. The inclusion criteria included patients aged 18-59 years with mild symptoms who had their serum 25(OH)D levels assessed within two months before infection and who received treatment at the Foresta Clinic. The sample size was calculated using a formula for comparing two independent means, based on research by Filippo LD et al.,[10] with an α at 0.05 and a β at 0.20. An additional 10% was added to the calculated sample size, resulting in 85 patients per group, for a total of 170 patients in the study.

The psychiatric symptoms of long COVID encompassed anxiety, depression, and sleep disorders, which were assessed using a numerical rating scale ranging from 0 to 10 points (0 indicating signs absent or no change compared to prior infection and 10 indicating the most severe symptoms). We adapted this questionnaire from the Department of Medical Services' Long-Term Health Impact Questionnaire for COVID-19 Survivors, which had a Kuder-Richardson coefficient of reliability of 0.8638.

The instruments used in this study comprised a data collection form divided into three parts: demographic data, vitamin D levels, and psychiatric symptoms of long COVID. We collected the demographic data and psychiatric symptoms section using self-administered questionnaires. Demographic data included gender, age, and body mass index (BMI). The psychiatric symptoms of long COVID encompassed anxiety, depression, and sleep disorders, which were assessed using a numerical rating scale ranging from 0 to 10 points (0 indicating no symptoms and 10 indicating the most severe symptoms). This scale was adapted from the Long-Term Health Impact Questionnaire for COVID-19 patients developed by the Department of Medical Services. We obtained serum 25(OH) D from the clinic's medical database and measured it in ng/mL. These levels were categorized according to the guidelines of the Endocrine Society of the United States into three levels: vitamin D deficiency (<20 ng/mL), insufficient vitamin D levels (20–30 ng/mL), and sufficient vitamin D levels (> 30 ng/mL).

Statistical Analysis

We used descriptive statistics to present demographic data and psychiatric symptoms associated with long-term COVID. For continuous data, the mean, standard deviation (SD), median, and interquartile range (IQR) were calculated, while categorical data were summarized using frequencies and percentages. The demographic data of patients were compared across three groups based on their vitamin D levels using one-way ANOVA and the chi-square test. We used the Mann-Whitney U test to assess the relationship between vitamin D levels and the presence of psychological symptoms in long-term COVID. We conducted data analysis using STATA statistical software (StataCorp. Stata Statistical Software: Release 18. College Station, TX: StataCorp LLC; 2023). All tests were two-tailed. Statistical significance was set at the 0.05 level ($\alpha = 0.05$).

Results

Demographic data were analyzed from 170 COVID-19 patients, showing a female-to-male ratio of 1.1:1. The mean age was 45.87 ± 8.65 years. The mean height was 162 ± 6.07 cm, and the mean weight was 65.42 ± 11.48 kg. The mean body mass index (BMI) was 24.90 ± 4.72 kg/m^2, with the majority being classified as overweight (62.1%). Table 1 shows no statistically significant differences in the demographic factors across the three vitamin D level groups ($p > 0.05$).

Table 1 Demographic data of COVID-19 patients

	Total (n = 170)	Vitamin D status			*p*-value
		Deficiency (n = 52)	Insufficiency (n = 70)	Sufficiency (n = 48)	
Sex, n (%)					
Male	83 (48.8)	26 (50.0)	34 (48.6)	23 (47.9)	0.977
Female	87 (51.2)	26 (50.0)	36 (51.4)	25 (52.1)	
Age (years), mean ± SD	45.87 ± 8.65	45.85 ± 8.87	45.17 ± 8.85	46.92 ± 8.18	0.563
Height (cm), mean ± SD	162 ± 6.07	163.12 ± 6.58	162.06 ± 5.94	162.21 ± 5.73	0.612
Weight (kg), mean ± SD	65.42 ± 11.48	65.94 ± 11.37	64.03 ± 11.30	66.88 ± 11.85	0.387
BMI (kg/m^2), mean ± SD	24.90 ± 4.72	24.87 ± 4.55	24.48 ± 4.67	25.54 ± 5.01	0.489
< 18.5	17 (10.1)	4 (7.8)	8 (11.4)	5 (10.4)	
18.5 – 22.9	47 (27.8)	16 (31.4)	20 (28.6)	11 (22.9)	0.866
> 23.0	105 (62.1)	31 (60.8)	42 (60.0)	32 (66.7)	

Data were analyzed with chi-square test and one-way ANOVA; * statistically significant at the 0.05 level ($\alpha = 0.05$)

The prevalence of psychiatric symptoms was 30.0% (95% CI 23.2, 37.4). These symptoms included anxiety at a rate of 15.3%, with a mean severity level of 3.12 ± 1.07; depression at 7.1%, with a mean severity level of 2.67 ± 1.78; and sleep disorders at 21.2%, with a mean severity level of 4.44 ± 2.08 (Table 2).

Table 2 Prevalence and severity of psychiatric symptoms in Long COVID patients

Psychiatric symptoms	n (% [95%CI])	Severity score, mean ± SD
Overall psychiatric symptoms	**51 (30.0 [95%CI 23.2, 37.4])**	
Anxiety	26 (15.3)	3.12 ± 1.07
Depression	12 (7.1)	2.67 ± 1.78
Sleep disorder	36 (21.2)	4.44 ± 2.08

The study on how common psychiatric symptoms is in long COVID patients related to their vitamin D levels showed that patients lacking vitamin D had the most psychiatric symptoms (46.2%), followed by those with low vitamin D (25.7%) and those with enough vitamin D (18.8%). These differences were statistically significant at the 0.05 level ($p = 0.007$). In the same way, patients with vitamin D deficiency had a much higher rate of anxiety, depression, and sleep disorders than those with insufficient or sufficient vitamin D levels, and this was statistically significant at the 0.05 level (Table 3).

Table 3 Prevalence of psychiatric symptoms according to vitamin D status

Psychiatric symptoms	Vitamin D status			*p*-value
	Deficiency (n = 52)	Insufficiency (n = 70)	Sufficiency (n = 48)	
Overall psychiatric symptoms	**24 (46.2)**	**18 (25.7)**	**9 (18.8)**	**0.007***
Anxiety	14 (26.9)	8 (11.4)	4 (8.3)	0.018*
Depression	10 (19.2)	2 (2.9)	0 (0.0)	< 0.001*
Sleep disorder	21 (40.4)	10 (14.3)	5 (10.4)	< 0.001*

Data was analyzed with chi-square test; * statistically significant at the 0.05 level ($\alpha = 0.05$)

Discussion

This study found a prevalence of psychiatric symptoms in 30.0% of patients (95% CI 23.2, 37.4), divided into 15.3% with anxiety, 7.1% with depression, and 21.2% with sleep disorders. In several intriguing aspects, the findings of this study are both consistent with and different from other studies. This study can be compared to the systematic review and meta-analysis conducted by Kokolevich ZM, et al.[11] which revealed a similar prevalence of anxiety and sleep disorders, with 14.5% for anxiety and 20.0% for sleep disorders. This consistency may reflect general trends in psychiatric symptoms among patients. The systematic review and meta-analysis by Gennaro FD et al.[6], which included data from 120,970 patients, showed a slightly lower overall prevalence of psychiatric symptoms (20.3%) compared to this study. However, the rates of anxiety (18.9%) and sleep disorders (17.8%) were comparable, while depression had a higher prevalence (16.1%). This discrepancy may be attributed to the diversity of populations and variations in data collection methods. In contrast, a study in China found a significantly higher prevalence of anxiety (30.2%) and sleep disorders (29.2%).[12] Cultural, social, or environmental factors specific to China may account for this difference. A study conducted in Thailand with 364 patients found a prevalence of psychiatric symptoms of 21.3%, which was lower than this study.[13] This difference may be due to several factors, such as sample size, study period, or differing characteristics of the population. It is evident that psychiatric symptoms, particularly anxiety and sleep disorders, are common problems in patients, consistent with studies from various countries, despite some differences in details. The variation between studies may reflect differences in populations, data collection methods, and distinct cultural and social factors.

A study comparing vitamin D levels in patients with and without psychiatric symptoms from long COVID found that

those with symptoms like anxiety, depression, and sleep problems had much lower average blood vitamin D levels than those without these symptoms, and this difference is statistically significant at the 0.05 level. The results suggest that levels of vitamin D are associated with the psychiatric symptoms in long COVID, including anxiety, depression, and sleep disorders. Patients with vitamin D deficiency are 3.86 times more likely to develop psychiatric symptoms compared to those with sufficient levels of vitamin D. This information is consistent with the study by Algin S, et al,[14] which reported a mean vitamin D level of 19.9 ± 11.8 ng/mL in patients with mental disorders, with low vitamin D levels as high as 87.8%. Of these, 62.0% had levels below 20 ng/mL, and 25.8% had levels between 21 and 30 ng/mL. Similarly, the study by Hikmet RG, et al,[15] found that 89.0% of individuals with low vitamin D levels experienced sleep disorders.

Vitamin D not only plays a vital role in serotonin synthesis but also supports the function of nerve cells, protects neurons, and affects neurotransmitters essential for sensory perception, cognitive function, and emotional control. Neurological symptoms like headaches, dizziness, and sensory loss are common in long COVID patients and are thought to be caused by the virus's impact on the nervous system. A deficiency of vitamin D can hinder neuropsychiatric recovery in these patients. When vitamin D levels are low, it can increase inflammation in the nervous system, potentially damaging the cranial and olfactory nerves. This damage may worsen symptoms such as dizziness, headaches, and loss of smell or taste.

This finding matches a study by Algin et al. that showed patients with psychiatric disorders had an average vitamin D level of 19.9 ± 11.8 ng/mL, with 87.8% of them having low vitamin D levels, including 62.0% with levels below 20 ng/mL and 25.8% with levels between 21 and 30 ng/mL. Vitamin D plays a crucial role in mental health and the nervous system, particularly in the context of long COVID.

A deficiency in vitamin D can affect the synthesis of neurotransmitters, particularly serotonin, which plays a crucial role in regulating mood. Moreover, vitamin D has protective properties for the nervous system, helping to shield brain cells from damage and supporting brain development and function. Vitamin D deficiency may contribute to neurological disorders that affect cognition and mood. In long COVID, chronic inflammation is a key factor linking psychiatric symptoms. Vitamin D helps modulate the immune response by reducing the levels of pro-inflammatory cytokines. A deficiency in vitamin D can make inflammation worsen, negatively affecting brain function and leading to emotional disturbances. Vitamin D is also involved in regulating the hypothalamic-pituitary-adrenal (HPA) axis, which plays a critical role in the response of stress. Vitamin D sufficiency can promote the HPA axis and decrease the risk of mental health issues.

Vitamin D plays a crucial role in mental health and the nervous system, particularly in the context of long COVID. It may influence neurotransmitter synthesis, especially serotonin, which affects mood regulation and increases the risk of depression and anxiety. Additionally, vitamin D has neuroprotective properties, helping to shield brain cells from damage and supporting the brain's development and function. Therefore, a deficiency in vitamin D could lead to neurological disorders, affecting both cognitive function and mood.[16] In the case of long COVID, chronic inflammation is a significant factor linked to psychiatric symptoms. Vitamin D helps modulate immune responses by reducing the levels of cytokines that promote inflammation. A deficiency in vitamin D

could cause severe inflammation, affecting brain function and leading to mood disorders.[17] Furthermore, vitamin D is involved in regulating the hypothalamic-pituitary-adrenal (HPA) axis, which plays a critical role in stress response. A deficiency in vitamin D may impair the functioning of the HPA axis, increasing the risk of mental health issues.[18] Moreover, studies on the effectiveness of vitamin D supplementation in randomized controlled trials have reported that receiving 60,000 IU of vitamin D per week significantly reduces anxiety, though it does not appear to alleviate depression.[19] However, some studies have found no association between vitamin D levels and symptoms of anxiety, depression, or sleep disorders.[15]

The reason for studying how vitamin D affects long COVID is that vitamin D is important for controlling inflammation in the body by both promoting and reducing it. Vitamin D deficiency can lead to chronic inflammation, which is common in long-term COVID patients who continue to experience inflammation even after recovering from the infection, resulting in persistent fatigue —for example, chronic fatigue syndrome and brain fog. Additionally, vitamin D is important for energy production and mitochondrial function. When low vitamin D levels decrease mitochondrial efficiency, leading to reduced energy production in the body, which can manifest as fatigue. Another symptom commonly seen in long COVID is fever, which is a general response to infection and inflammation triggered by the release of cytokines. Low vitamin D levels may lead to the continuous release of cytokines, causing prolonged or recurrent fever. On the other hand, maintaining sufficient vitamin D levels can help reduce the production of pro-inflammatory cytokines and diminish free radicals. In summary, sufficient vitamin D levels are important for regulating inflammation, mitochondrial function, and cytokine production. These processes are not only associated with chronic fatigue but also with neuropsychiatric symptoms via the regulation of neurotransmitter synthesis. When it comes to the possible advantages of vitamin D for mental health, vitamin D is important for making neurotransmitters, helps nerve cells work properly, protects neurons, reduces harmful substances like tau and beta-amyloid proteins, and influences neurotransmitters that are crucial for how we perceive things, think, and manage our emotions.

Vitamin D also contributes to sleep disorders, as brain regions that regulate sleep contain vitamin receptors. It is believed that vitamin D influences the production of melatonin, a hormone that regulates the sleep-wake cycle. Vitamin D deficiency may disrupt melatonin production, leading to sleep disorders.[20] Even in patients with long COVID, vitamin D deficiency could worsen symptoms, increasing stress in managing the condition. Such conditions can lead to deteriorating mental health, difficulties in emotional regulation, heightened anxiety, and sleep disorders.[21]

This study suggests that vitamin D levels are associated with psychiatric symptoms in long COVID patients. Therefore, healthcare professionals should prioritize monitoring and assessing vitamin D levels in this group of patients, as it may have the potential to prevent and reduce the severity of psychiatric symptoms. Additionally, vitamin D supplementation should be considered for those with a deficiency. Further research is needed to evaluate the efficacy of vitamin D supplementation in preventing or decreasing psychiatric symptoms in long COVID patients, particularly regarding anxiety, depression, and sleep disorders. Moreover, cultural, social, and environmental factors that may influence patient responses should

be considered. Health care should adopt a holistic care approach, addressing physical, psychological, and nutritional factors to develop effective and appropriate treatment strategies for each individual. However, our findings showed that vitamin D status and psychiatric disorders are independent of BMI.

Conclusion

This study identified a significant association between vitamin D levels and psychiatric symptoms in patients with long COVID, particularly in relation to anxiety, depression, and sleep disorders. The findings indicate that patients exhibiting psychiatric symptoms often have lower vitamin D levels. This vitamin D deficiency may negatively impact nervous system function, immune response, inflammatory processes in the body, stress regulation, and the production of hormones associated with the sleep-wake cycle, all of which can affect the mental health of patients. Health care should also consider other factors, such as cultural, social, and environmental, which may affect the manifestation and response to psychiatric symptoms.

Acknowledgments

This study was partially supported by the MAS Neuroscience Research Center, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand. We sincerely thank all the volunteers who participated in this study. We would also like to thank the reviewers for their comments and suggestions.

Contribution

Conceptualization: Karn Matangkha and Jarasphol Rintra; Methodology: Karn Matangkha and Jarasphol Rintra; Formal analysis: Karn Matangkha and Jarasphol Rintra; Investigation: Vichit Punyahotara and Phakkharawat Sittiprapaporn; Writing-original draft preparation: Karn Matangkha and Phakkharawat Sittiprapaporn; Writing-review and editing, Phakkharawat Sittiprapaporn; Project administration: Jarasphol Rintra and Vichit Punyahotara. All authors have read and agreed to the published version of the manuscript.

Disclosure of interest

The authors report no conflict of interest.

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The Clinical Study of Poly-D, L-Lactic Acid (PDLLA) Biostimulator Injection for Improving Facial Rejuvenation Markers in Young Thai Individuals

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Received 23 April 2025 • Revised 2 June 2025 • Accepted 19 June 2025 • Published online 1 September 2025

Abstract:

Background: Aging is an inescapable and complex biological phenomenon that affects the skin, leading to changes such as wrinkles, sagging, pigmentation irregularities, and decreased elasticity. The latest trend in biostimulators has had a significant impact on facial rejuvenation, with poly-D, L-lactic acid (PDLLA) standing out. However, clinical trials on its long-term effects and safety remain limited, especially in Thailand.

Objective: The objective of this study was to investigate the effects of PDLLA injection in young Thai individuals for facial rejuvenation.

Materials and Method: In this quasi-experimental design, fifteen participants with mild to moderate facial aging signs enrolled at Mae Fah Luang University Hospital Asoke, Bangkok, Thailand. Each participant underwent subdermal PDLLA injections over a six-month period. Skin quality was evaluated at baseline and at 2, 4, and 6 months using validated instruments that measured eight facial rejuvenation parameters including sebum level, elasticity, skin hydration, transepidermal water loss (TEWL), spots, pores, wrinkles and texture. The study also evaluated the Global Aesthetic Improvement Scale (GAIS), patient satisfaction scores and any treatment-related side effects.

Results: All 15 participants showed significant improvement in elasticity, skin hydration, transepidermal water loss, pore size, and wrinkles from baseline to 6 months ($p < 0.001$). However, no significant differences were observed in sebum levels, spots, or texture. Mild skin erythema was observed in only one case (6.67%), which was spontaneously reversible over time.

Conclusion: Our study shows that PDLLA offers promising results for facial rejuvenation and can be an alternative for skin aging prevention, especially in young individuals, with no serious side effects when proper injection and reconstitution techniques are followed.

Keywords: Poly-D, L-lactic acid, Elasticity, TEWL, Hydration, Pore

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GMSMJ 2025; 5 (3): 159-168

Introduction

Facial rejuvenation treatments have gained significant attention in recent years, particularly among younger individuals seeking non-invasive solutions to maintain a youthful appearance. Beauty concerns related to aging, such as loss of facial volume and the appearance of fine lines, have become prevalent even among younger populations due to environmental factors, lifestyle choices, and genetics.[1] Thailand, like many countries globally, is experiencing a shift toward an aging population. According to national data, the proportion of individuals aged 60 and above in Thailand is 20 percent and is steadily increasing[2], leading to an increasing demand for preventive aesthetic treatments aimed at delaying or reversing the early signs of aging. Poly-D, L-lactic acid (PDLLA), a synthetic polymer derived from corn and potato starch[3-4], has emerged as a leading biostimulator in aesthetic medicine due to its ability to promote collagen synthesis through an inflammatory process, as proven by *in vitro* and *in vivo* studies.[5-7] PDLLA is biodegradable, with a study of PDLLA screws used in knee surgery showed complete resorption with intact surrounding tissue after 22 months.[8]

Although PDLLA has been widely studied and used in aging populations[7,9-10], there is limited research on its effectiveness in younger individuals, particularly in Thai populations. Younger individuals often seek preventative treatments that maintain or delay visible signs of aging, rather than reversing established signs. Understanding the impact of PDLLA injections on facial rejuvenation markers in young individuals is crucial for establishing its role in addressing the root causes of aging through early-stage aesthetic interventions. Therefore, this study aims to investigate the effects of PDLLA biostimulator injections on key facial rejuvenation markers, including sebum level, elasticity, skin hydration, transepidermal water loss (TEWL), spot, pore, wrinkle, and texture, in young Thai individuals. The outcomes will provide insights into the safety, efficacy, and long-term benefits of PDLLA as a preventive treatment, contributing valuable information to the growing field of aesthetic medicine.

Materials and Method

Study design and Participants

A quasi-experimental design was conducted for a before-and-after design from May 2024 to January 2025, recruiting 15 participants at Mae Fah Luang University Hospital Asoke, Bangkok, Thailand.

Sample size calculation

The sample size was calculated based on a study of Bohnert K, et al.[11] N4Studies Software, Version 1.4.0, was used to calculate the sample size. Estimated sample sizes were determined using a two-dependent means formula[12], based on a 5% α error, 80% power, and a 95% confidence level. The required number of patients for the study was calculated to be 15 after adjusting for a 20% dropout rate.

Method

The included participants were either male or female aged between 30 to 45 years with mild to moderate facial aging signs. We excluded individuals with poor medical conditions, active skin diseases, pregnancy or breastfeeding, and those with a history of biostimulator use. The study protocol was approved by the Ethics Committee on Human Research of Mae Fah Luang University (approval no. COA 97/2024). The scope of the work was explained to all participants and informed consent was obtained before participating in the study.

Materials

Study participants received injections of poly-D, L-lactic acid (PDLLA), commercially known as AestheFill (REGEN Biotech, Seoul, Korea), administered in two sessions spaced two months apart, as collagenesis typically reaches its peak around this time.[6] The Korean Food and Drug Administration granted initial approval for this product in 2014.[6] It also received approval from the Thai Food and Drug Administration (TFDA) under registration number 66-2-1-2-0004634. The product was supplied as a freeze-dried powder in vials, each containing 200 mg, composed of 154 mg of PDLLA and 46 mg of carboxymethyl cellulose (CMC). Prior to injection, 8 mL of sterile water was added to the PDLLA vial. Immediately before administration, an additional 2 mL of 2% lidocaine without adrenaline was incorporated, yielding a final dilution of 10 mL. The back-and-forth technique was utilized to ensure thorough mixing.[13] Local anesthesia (0.2 mL per site) was administered at the pre-hole, positioned along an imaginary line between the mid-preauricular and lateral canthus lines. Injections were delivered using a multilayer injection technique. A 23G blunt cannula was inserted at an angle of 30 to 40 degrees. A 23G cannula was chosen for its balance between flexibility and precision, minimizing tissue trauma and reducing the risk of bruising. Each injection line received 0.5 mL, with a total of five lines per side. Each participant received up to 2.5 mL of PDLLA per side, amounting to 5 mL per session.

Data collection

The assessments were conducted at specific time points (0 month, 2 months, 4 months, and 6 months). Demographic data, including age, gender, underlying disease, occupation, and personal history were collected. Data of the facial rejuvenation markers including the Sebum Level as measured by Sebumeter®[14], Elasticity as measured by Cutometer®[15], Skin Capacitance as measured by Corneometer®[16], Transepidermal Water Loss (TEWL) as measured by Tewameter®.[17]

Before each measurement, the Sebumeter® was zeroed by measuring the initial transparency of the tape without sebum present to ensure accuracy.[14] The Cutometer® was cleaned with a special brush and calibrated using a check cap per on-screen instructions.[15] The Corneometer® was calibrated *in vitro* using filter paper saturated with saline to simulate high hydration and a polyurethane film to represent low hydration, ensuring the device operated within validated measurement ranges.[16] The Tewameter® was calibrated using a check cap placed over the probe to create a sealed environment, allowing internal vapor concentration to be compared against skin measurements to detect and correct any drift.[17]

The first measurement site was identified at the intersection of the mid-pupil line and mid-tragus line, while the other four sites were positioned 1 cm above, below, left, and right of this point. The investigators performed each measurement five times on each side at every follow-up, totaling ten measurements per follow-up.

In addition, another four parameters of facial rejuvenation markers were assessed using the Visia-CR.[18] The participant was positioned in the machine, which automatically captured images and analyzed spots, pores, wrinkles, and texture, measured in arbitrary units (a.u.). Participants' satisfaction with the PDLLA injection was collected at the end of the study using a five-point Likert scale, while side effects were assessed using a side effect record form.

Statistical analysis

Data was analyzed using SPSS version 28.0 with *p*-value of less than 0.05 was considered statistically significant. The demographic data were presented as mean ± SD, while categorical data were expressed as frequency. The Shapiro-Wilk test was used to assess the normality of the data. Facial rejuvenation markers were analyzed using ANOVA with repeated measures for normally distributed data, while the Friedman test was applied when normality assumptions were violated. Participant satisfaction and side effects were reported as percentages.

Results

Demographic data of the 15 female participants (Table 1) showed an average age of 36.33 ± 3.85 years. Most had Fitzpatrick skin types 3 or 4 and no underlying conditions. None had allergies, took medications, or smoked, though some consumed alcohol. Participants came from various occupations, with housekeepers being the most common.

Throughout the 6-month study, notable changes in mean differences were observed. Significant improvements in Elasticity, Hydration, TEWL, Pore, and Wrinkle markers began at the 2-month mark and continued to increase over time, with the most significant difference noted from baseline to 6 months (-0.11 ± 0.02, $p < 0.001$). Conversely, Sebum levels, Spot, and Texture markers showed no significant changes, as outlined in Table 2.

At the end of the study, participants were asked to rate their overall satisfaction. The majority (93.3%) reported being extremely satisfied, while 6.67% were satisfied. The clinical changes were captured using VISIA-CR photographs in Figure 1. No major side effects occurred during the study. Throughout the study, no significant side effects were observed. The only reported issues were mild localized irritation, such as erythema (6.67%), which resolved on its own over time without any intervention.

Table 1 Demographic data of the 15 participants

Characteristics	Mean ± SD
Age (year)	36.33 ± 3.85
	Number of case
Sex	
Male	0
Female	15
Occupation	
Housekeeper	5
Therapist	3
Nurse	2
Office Employee	5
Business Owner	0
Unemployed	0
Fitzpatrick Skin Type	
Type 3	5
Type 4	10
Other	0
Underlying Disease	
None	15
Dyslipidemia	0
History of Food/Drug Allergy	0
Current Medication (Antibiotic, NSAIDs)	0
Current Smoker	0
Alcohol drinking	3

Table 2 The mean of facial rejuvenation markers at baseline, 2 months, 4 months, and 6 months (n = 15) with repeated measures ANOVA results

Markers	Baseline to 2 months (Mean Diff ± SD)	Baseline to 4 months (Mean Diff ± SD)	Baseline to 6 months (Mean Diff ± SD)	2 months to 4 months (Mean Diff ± SD)	2 months to 6 months (Mean Diff ± SD)	4 months to 6 months (Mean Diff ± SD)
Elasticity	-0.07 ± 0.02*	-0.16 ± 0.04**	-0.20 ± 0.03***	-0.09 ± 0.02***	-0.13 ± 0.02***	-0.04 ± 0.01***
Sebum level	0.01 ± 0.04	0.13 ± 0.08	-0.01 ± 0.26	0.11 ± 0.06	-0.03 ± 0.27	-0.14 ± 0.26
Hydration	-3.96 ± 0.98**	-8.76 ± 1.69***	-11.33 ± 1.65***	-4.80 ± 1.06***	-7.38 ± 1.11***	-2.58 ± 0.50***
TEWL	4.29 ± 1.31*	8.91 ± 1.14***	10.47 ± 1.17***	4.63 ± 0.73***	6.18 ± 0.79***	1.56 ± 0.27***
Spot	-0.08 ± 0.07	0.70 ± 0.11	0.26 ± 0.13	0.15 ± 0.07	0.33 ± 0.12	0.19 ± 0.11
Pore	3.21 ± 0.99*	8.43 ± 1.42***	9.90 ± 1.42***	5.21 ± 0.84***	6.69 ± 0.92***	1.47 ± 0.29***
Wrinkle	3.83 ± 1.06*	7.53 ± 1.18***	11.10 ± 1.43***	3.70 ± 0.99***	7.27 ± 1.35***	3.57 ± 0.73***
Texture	0.04 ± 0.06	0.26 ± 0.12	-0.22 ± 0.26	0.23 ± 0.11	-0.25 ± 0.26	-0.48 ± 0.28

Note Data were analyzed using Repeated measure ANOVA followed by Bonferroni post hoc tests, SD for (Standard Deviation), Mean diff for (Mean Difference), TEWL for (Transepidermal Water Loss), Values with different superscript symbols (*, **, ***) indicate statistical significance at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

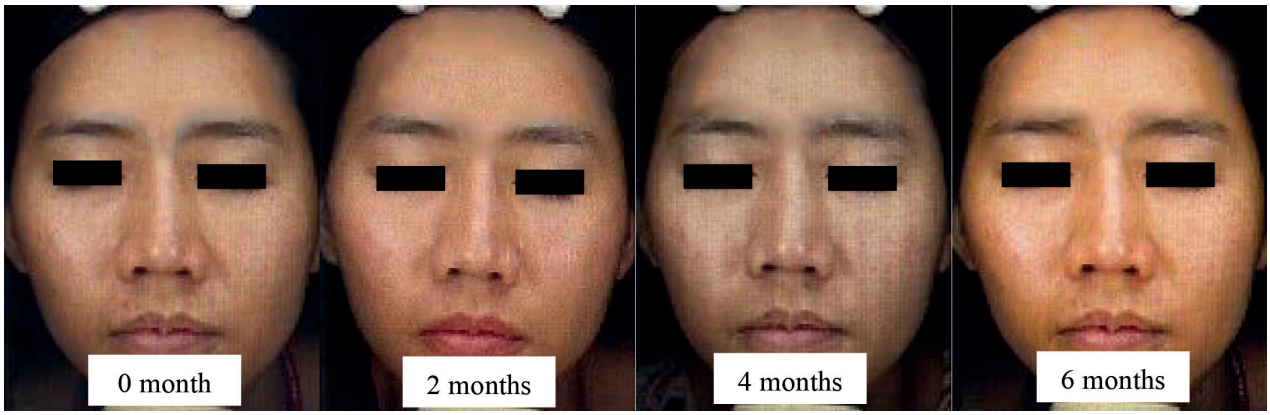

Figure 1 The comparison of VISIA-CR photographs of representative case at 0, 2, 4, and 6 months, respectively

Discussion

The present study aimed to evaluate the efficacy of poly-D, L-lactic acid (PDLLA) as a biostimulator for facial rejuvenation, focusing on various markers. The findings not only highlight the potential benefits of PDLLA in enhancing facial aesthetics but also contribute to the existing body of literature on biostimulator therapies.

In terms of effectiveness, the data indicated a statistically significant improvement in skin elasticity, hydration, TEWL, pore size, and wrinkles among participants receiving PDLLA treatment, starting from the second month. The early changes, particularly in hydration, may be attributed to the immediate filling effect post-injection. However, this effect diminishes after approximately two weeks, with subsequent improvements likely resulting from collagen synthesis.[5]

Our study could be applied to other biostimulator indications. For instance, Lin and Lin (2022) reported improved skin elasticity and hydration after a single-session PDLLA (AestheFill) injection for under-eye rejuvenation, using a 4 mL formulation (3 mL SWFI and 1 mL lidocaine) with 2 mL per side.[9] Given the delicate nature of the periorbital area, adapting our midface injection and dilution technique—such as using a 23G cannula for controlled, precise subdermal deposition—could further optimize outcomes while minimizing risks in periorbital treatments.

Likewise, a recent study by Seo et al. (2024) highlighted the use of PDLLA (Juvelook) for skin rejuvenation with a mesotherapy injector equipped with 32G 9-pin needles. The formulation, comprising hyaluronic acid (HA), lidocaine, and saline, was administered over three sessions in a study involving 16 participants and demonstrated promising results.[10] Given PDLLA's particle size (30–60 μm), the use of a 23G cannula, as in our study, provides advantages such as smoother delivery, reduced clogging, and controlled subdermal deposition, while also minimizing trauma, lowering vascular injury risk, and enhancing patient comfort. The selection of a 23G cannula is the reason why our study reported no bruising or serious side effects and achieved consistent clinical outcomes.

In terms of safety, Ianhez et al. (2024) reported complications from collagen biostimulators, with poly-L-lactic acid (PLLA) accounting for 69.1% of 55 cases, primarily nodules (89.1%), often persisting despite treatment.[19] PDLLA offers a safer alternative to PLLA, a similar chemical isomer. Bohnert et al. (2019) evaluated PLLA in three treatment sessions spaced four weeks

apart, requiring post-injection massage to prevent nodules.[11] Despite its efficacy, this additional step was necessary. In contrast, our study used PDLLA with a 23G cannula in only two sessions, achieving significant improvements in skin elasticity, hydration, and TEWL without massage. PDLLA may offer a safer alternative due to its smaller particle size, uniform degradation, and reduced nodule risk. PDLLA's smoother tissue integration eliminates the need for post-injection massage, unlike PLLA. Additionally, using a 23G cannula, as in our study, enhances safety by ensuring controlled placement and minimizing vascular injury. These differences underscore PDLLA's advantages, including a lower risk of nodules, fewer sessions, and controlled deposition, making it a promising option for facial biostimulation in young-aged individuals.

Our study introduces an innovative approach, highlighting the ideal candidates for PDLLA injection. These include the young-aged group with poor skin quality, such as loose skin, dry skin, large pore size, and fine wrinkles, particularly in the middle face area. Although PDLLA is safer than PLLA, there is a case report by Choi Min et al. (2024) of granulomas in the infraorbital area. A 58-year-old woman developed granulomas after her third PDLLA injection, which were unresponsive to TCA and light therapy, requiring surgery.[20] This emphasizes the importance of careful injection technique, especially in thin-skinned areas like the infraorbital region. Proper injection technique, including precise placement and appropriate dosage, is crucial to minimizing risks, as demonstrated in our study.

While the results of this study are promising, certain limitations should be acknowledged. This study's limited sample size and lack of participant or evaluator blinding may introduce potential bias and limit the generalizability of the findings. Although PDLLA injections have proven effective in facial rejuvenation, the study did not assess PDLLA's impact in combination with other procedures or on other body parts, where skin structure, thickness, and tissue response to substances may vary significantly. Future studies should explore PDLLA's impact on various skin types and demographics, as well as its combination with other procedures like laser treatments or microneedling. Expanding research to areas such as the neck, décolletage, and hands could provide further insights into PDLLA's efficacy and safety across different anatomical sites, optimizing clinical applications.

Conclusion

This study demonstrated that Poly-D, L-lactic acid (PDLLA) significantly improves facial rejuvenation markers, including skin elasticity, hydration, TEWL, pore appearance, and wrinkles over 6 months. Notable improvements were seen starting at 2 months, with continued progress after the second injection. PDLLA offers a safe alternative for skin aging prevention in young individuals, with no serious side effects when proper techniques are followed.

Acknowledgements

The authors would also like to express their sincere gratitude to the staffs at Mae Fah Luang University Hospital Asoke, Bangkok, Thailand, for their invaluable support with data collection.

Conflict of interest

The authors declare no conflicts of interest.

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Short Communication GMSMJ Greater Mekong Subregion Medical Journal

Association between Antimicrobial Administration and Nasal Carriage of *Pseudomonas aeruginosa* and *Candida* Species in ICU Patients

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Received 21 April 2025 • Revised 26 April 2025 • Accepted 30 May 2025 • Published online 1 September 2025

Abstract:

Background: Patients admitted to the intensive care unit (ICU) often develop hospital-acquired infections (HAI).

Objective: We started an active surveillance culture (ASC) to monitor bacterial or fungal carriage in the ICU of our hospital and retrospectively evaluated in this study.

Materials and Method: ASC was performed using a nasal swab culture when the patients were admitted to the ICU, regardless of whether the patients had any infectious diseases. If the patients continued to stay in the ICU for the following week or later, ASC was performed regularly once a week until discharge.

Results: When comparing the bacteria isolated from nasal swab cultures at the time of ICU admission, *Pseudomonas aeruginosa*, which sometimes develops drug resistance and can cause HAI, was isolated more frequently from ASC the following factors for increased isolation of *Pseudomonas aeruginosa* week or later. Antipseudomonal penicillin and antifungals were independent risk for increased isolation of *Candida* species. Considering that most ICU patients are administered antimicrobials, it was suggested that using antimicrobials during long-term ICU stays affects the nasal bacterial flora of ICU patients.

Conclusion: ASC would help understand the nasal carriage status and changes in the bacterial flora of ICU patients, supporting taking measures against drug-resistant bacteria.

Keywords: Antimicrobial, *Pseudomonas aeruginosa*, *Candida* species, ICU

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GMSMJ 2025; 5 (3): 169-179

Introduction

Patients admitted to intensive care units (ICU) are relatively immunocompromised due to their severe illness. They are constantly exposed to the risk of infection due to the insertion of various medical devices or in vasive treatments such as surgery. In addition, ICU patients often develop hospital-acquired infections (HAI), including ventilator-associated pneumonia, central venous catheter-associated bloodstream infections, and catheter-associated urinary tract infections. Such severe or life-threatening infectious diseases are common among ICU patients, and most of them are caused by bacterial or fungal infection and require antimicrobial therapy for clinical resolution.[1-4] These patients are prone to opportunistic infections caused by endogenous or environmental bacteria. In addition, antimicrobials are administered to most ICU patients to treat infections or preventive purposes, such as perioperative administration.[5] The Extended Prevalence of Infection in Intensive Care (EPIC II) study, which investigated the status of infections in ICUs in 75 countries, reported that 51% of ICU patients had infectious diseases.[6] In addition, the Japanese Survey of Antimicrobial Use in ICU Patients (JSCRIPT) study, which investigated antimicrobial use in Japan, found that 50.1% of ICU patients had some bacterial infection, and 72.6% were administered intravenous antimicrobials. Empirical antimicrobial treatment was started in 43.1% of patients, and antimicrobials were administered prophylactically to 32.8% of patients.[7] Therefore, the selective pressure by antimicrobial administration increases the risk of ICU patients carrying resistant bacteria. Since ICU patients are always relatively immunocompromised, increasing the pressure of carrying resistant bacteria is a risk factor for transmitting infection to other patients. Active surveillance culture (ASC) is often performed as a screening method for patients with resistant bacteria. We reported that administering antimicrobials was a significant risk factor that affects changes in the nasal bacterial flora of ICU patients.[8] In particular, glucose non-fermenting gram-negative rods (NF-GNR), including *Pseudomonas aeruginosa*, and *Candida* species, increased after admission to the ICU.

In this study, we investigated factors that affect changes in the isolation status of *Pseudomonas aeruginosa* and *Candida* species in the ASC of ICU patients.

Subjects and Method

This study is a retrospective, single center-cohort study and included a total of 815 ASC samples submitted from 366 patients who were admitted to the ICU of Yamagata Prefectural Central Hospital from June 2015 through September 2016. All of the 366 patients underwent ASC by nasal swab culture at the time when admitted to the ICU, regardless of whether they had any infectious diseases (Initial ASC). If the patient continued to stay in the ICU from the following week onwards, ASCs were continued once a week regularly until discharge (Follow-up ASC). Samples were collected from all ICU-admitted patients, with no cases of refusal. There were 364 actual patients who underwent the initial ASC, and 138 actual patients who underwent the follow-up ASC. The culture conditions for ASC were 35°C and 5% carbon dioxide

culture for sheep blood agar medium (Kyokuto Pharmaceuticals, Japan), and 35°C and aerobic culture for BTB agar medium (Kyokuto Pharmaceuticals, Japan), Chromoagar Candida medium (Kanto Chemicals, Japan), and Poremedia MRSA isolation medium (Eiken Chemicals, Japan) for MRSA screening. The cultures were observed after 24 and 48 hours, and the culture-positive bacterial species were identified using a fully automated bacterial testing device, VITEK2 (SYSMEX bioMérieux, Japan). Bacteria or fungi isolated from nasal swab cultures of the patients were counted as one for each strain, including cases in which multiple bacteria or fungi were isolated from the same patient simultaneously. In addition, each case was counted as one even if the culture was negative. The antimicrobial susceptibility of the isolated bacteria was determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

All isolated bacteria were classified according to the time of isolation into two groups: those isolated at the time of ICU admission (Initial ASC group) and those isolated every week after admission (Follow-up ASC group), and the clinical and bacteriological information was evaluated. Data was analyzed using JMP 14 (SAS Institute), with Wilcoxon tests for continuous variables and chi-square tests for non-continuous variables. Multivariate analysis was performed using multiple logistic regression analysis.

This study was reviewed and approved by the Yamagata Prefectural Central Hospital Ethics Committee (approval number: H30-25).

Results

A total of 815 ASC samples submitted from 366 patients who were admitted to the ICU during the period were analyzed. The initial ASC group, in which samples were collected from patients only at admission to the ICU, consisted of 569 samples (364 actual patients). The follow-up ASC group, in which samples were collected from patients in the ICU from the following week onwards, consisted of 246 samples (138 actual patients) (Table 1A and 1B).

When comparing the clinical background of patients in both groups, there were no significant differences in gender and age. Antimicrobials were used in the initial ASC group for 257 out of 364 cases (70.6%). Among these, 57 cases (15.7%) were complicated by infectious diseases. Seven of these 57 cases had antimicrobials administered more than one week before ICU admission, and 15 had antimicrobials administered within one week before admission. The remaining 35 cases had antimicrobials started simultaneously with admission. The 200 cases without infectious diseases had also antimicrobials started simultaneously with admission. While antimicrobials were used in 133 of 138 cases (96.4%), and any infectious diseases in the follow-up ASC group complicated 21 cases (15.2%) (Table 1A). When comparing the proportion of ASC among the isolated strains in both groups, the bacteria which consist of normal nasal flora, such as coagulase-negative Staphylococci (CNS) and Corynebacterium species, accounted for 64.5% (367 strains) in the initial ASC group whereas it was 43.5% (107 strains) in the follow-up ASC group (Figure 1).

Table 1A Patient characteristics

ICU stay	Initial ASC (≤1 week)	Follow-up ASC (>1week)	p-value
Patients, number	364	138	N/A
Gender, male, number (%)	243 (66.8%)	104 (75.4%)	0.059
Age, years, median (IQR)	71 (63-78)	70 (63.8-79)	0.42
ID co-morbidities, number (%)	57 (15.7%)	21 (15.2%)	0.90
ABX use, number (%)	257 (70.6%)	133 (96.4%)	< 0.0001*

ICU: Intensive care unit, ASC: Active surveillance culture, IQR: Interquartile range, ID: Infectious diseases, ABx: antimicrobials
*p < 0.05 as a statistically significant difference

Table 1B ASC profiles

ICU stay	Initial ASC (≤1 week)	Follow-up ASC (>1week)	p-value
ASC samples, number	569	246	N/A
Under ABX use, number (%)	386 (67.8%)	237 (96.3%)	< 0.0001*
Isolated microbes, number (%)	506 (88.9%)	233 (94.7%)	0.0062*

ASC: Active surveillance culture, ICU: Intensive care unit, ABx: antimicrobials
*p < 0.05 as a statistically significant difference

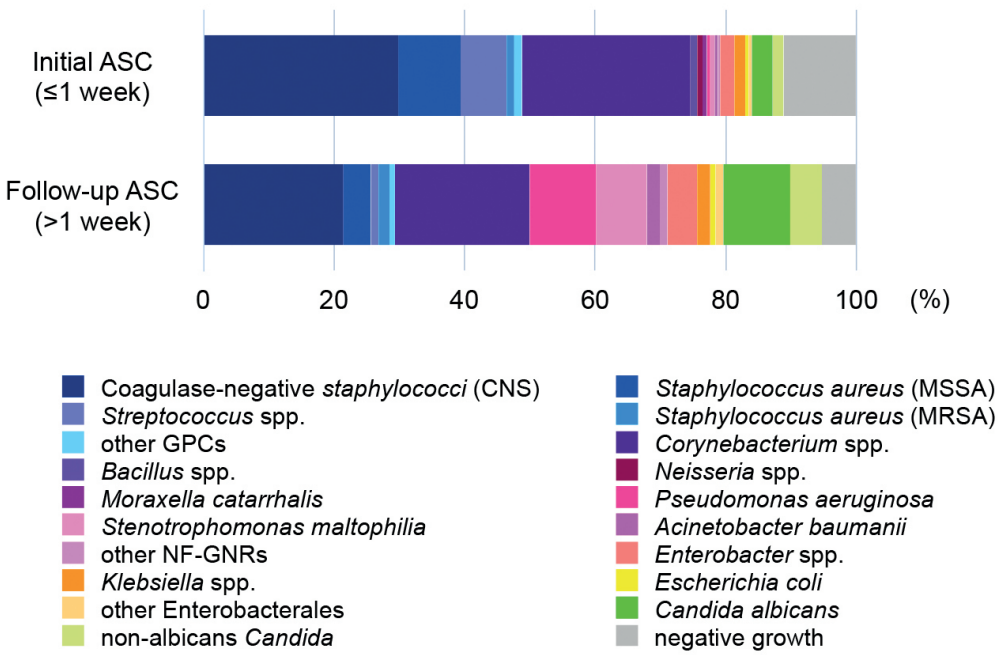

Figure 1 Proportion of isolated bacterial or fungal cultures

ASC: Active surveillance culture, MSSA: Methicillin-sensitive *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, GPC: Gram positive coccus, NF-GNR: Glucose non-fermenting gram-negative rods, spp: species

In terms of comparing the changes in isolated bacteria or fungi in both the initial ASC group and the follow-up ASC group, *Pseudomonas aeruginosa* was increased from 0.5% (3 strains) to 10.2% (25 strains) (Figure 1). Regarding the drug susceptibility of isolated *Pseudomonas aeruginosa*, the number of strains resistant to antipseudomonal drugs (I or R) was increased from 1 to 12 (Figure 2). In patients who had received some antimicrobials (carbapenems, fluoroquinolones, antipseudomonal penicillin, aminopenicillin, the first-generation cephalosporin, anti-MRSA drugs, and antifungals), the rate of isolation of *Pseudomonas aeruginosa* was increased during ICU stay, regardless of whether the initial ASC or the follow-up ASC (Table 2A). The effect of administration of these antimicrobials on isolation of *Pseudomonas aeruginosa* was examined by multivariate analysis, resulting in administration of antipseudomonal penicillin (odds ratio 5.76) and antifungals (odds ratio 9.75) were independent risk factors for isolation of *Pseudomonas aeruginosa* (Table 2B).

In addition, the number of *Candida* species in the initial ASC group increased from 4.9% (28 strains) to 15.0% (37 strains) in the follow-up ASC group. Of these, the number of *Candida albicans* cases increased from 18 to 25, but the number of non-albicans *Candida* cases did not change significantly, from 10 to 12 (Figure 3). Similarly, we investigated the effect of antimicrobial administration on the isolation of *Candida* species, and the isolation of *Candida* species was higher in patients who had received carbapenems, fluoroquinolones, aminopenicillins, or anti-MRSA drugs. However, no change was observed in patients who had received antipseudomonal penicillin, first-generation cephalosporin, or antifungals (Table 3A). Multivariate analysis showed that the administration of carbapenems was an independent risk factor for the isolation of *Candida* spices (odds ratio 2.11) (Table 3B).

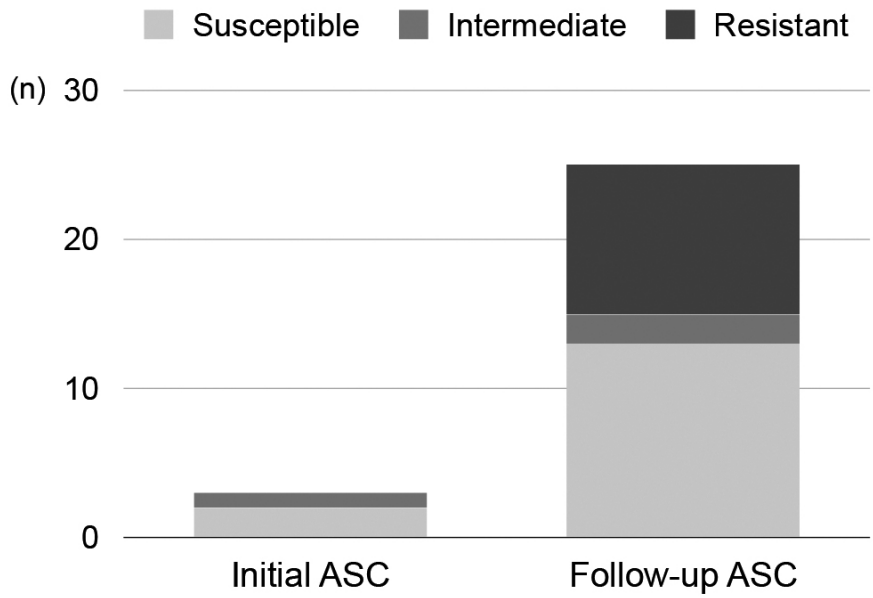

Figure 2 Prolonged ICU stay increased *P. aeruginosa* in ASC
ASC: Active surveillance culture

Table 2A Effect of ABx on *Pseudomonas aeruginosa* isolation in ASC

ASC	*P. aeruginosa*	others	p-value
ASC isolation, number	28	787	N/A
Carbapenems, number (%)	15 (53.6%)	152 (19.3%)	< 0.001*
Fluoroquinolones, number (%)	5 (17.9%)	33 (4.2%)	0.0082*
Antipseudomonal penicillins, number (%)	23 (82.1%)	329 (41.8%)	< 0.0001*
Aminopenicillins, number (%)	16 (57.1%)	145 (18.4%)	< 0.0001*
1st gen. Cephalosporins, number (%)	20 (71.4%)	358 (45.5%)	0.0063*
Anti-MRSA antibiotics, number (%)	6 (21.4%)	68 (8.6%)	0.0437*
Antifungals, number (%)	13 (46.4%)	44 (5.6%)	< 0.0001*
No ABX use, number (%)	1 (3.6%)	191 (24.3%)	0.0027*
Age, years, median (IQR)	71 (66-80)	70 (63-78)	0.3158
Gender, male, number (%)	22 (78.6%)	552 (70.1%)	0.3221

The isolation of *P. aeruginosa* during ICU stay, regardless of whether the initial ASC or the follow-up ASC, were evaluated.
Data was analyzed with Wilcoxon tests for continuous variables and chi-square tests for non-continuous variables.
Clinically important factors were evaluated using univariate analysis, followed by multivariate analysis adjusting for confounding factors.
ABx: antimicrobials, ASC: Active surveillance culture, 1st gen: First generation, MRSA: Methicillin-resistant *Staphylococcus aureus*, IQR: Interquartile range
*$p < 0.05$ as a statistically significant difference.

Table 2B Independent risk factors for isolation of *Pseudomonas aeruginosa* by administration of antimicrobial

	OR	95% C.I.	p-value
Antipseudomonal penicillins	5.76	1.49-23.57	0.0108*
Antifungals	9.75	1.66-89.29	0.0099*

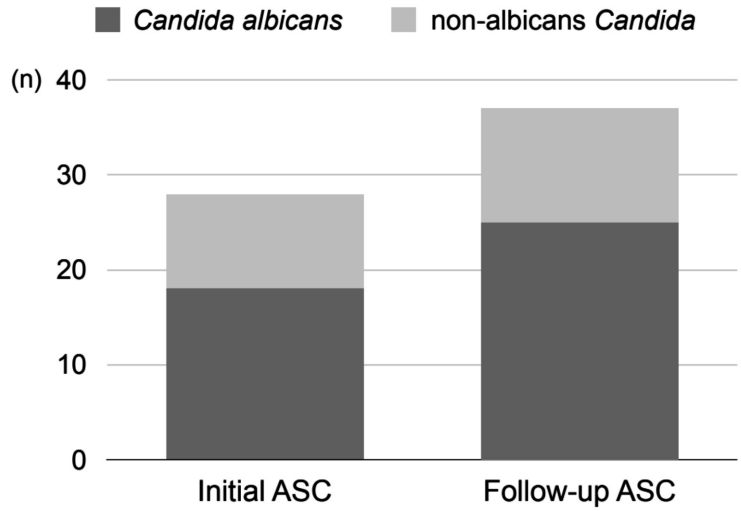

Figure 3 The number of *Candida* species in the initial ASC group increased from 4.9% (28 strains) to 15.0% (37 strains) in the follow-up ASC group

Discussion

Our results showed non-resident intranasal bacteria, such as Enterobacteriaceae, *Pseudomonas aeruginosa* and other NF-GNR, or *Candida* species, were increased in nasal ASC from the week after admission to the ICU. This result suggested that the nasal flora changed due to the extension of the ICU stay. It is known that an extended stay in the ICU increases the risk of acquiring resistant bacteria such as carbapenem-resistant gram-negative bacilli and MRSA.[9,10]

Table 3A Effect of ABx on *Candida* spp. isolation in ASC

	***Candida* spp. (n = 65)**	**others (n = 750)**	**p-value**
Carbapenems	26 (40.0%)	141 (18.8%)	0.0002*
Fluoroquinolones	0 (0%)	38 (5.1%)	0.0109*
Antipseudomonal penicillins	34 (52.3%)	318 (42.4%)	0.1238
Aminopenicillins	20 (30.8%)	141 (18.8%)	0.0273*
1st gen. Cephalosporins	31 (47.7%)	347 (46.3%)	0.8251
Anti-MRSA antibiotics	13 (20.0%)	61 (8.1%)	0.0045*
Antifungals	5 (7.7%)	52 (6.9%)	0.8204

Table 3B Risk factor for *Candida* spp. Isolation

	OR	**95% C.I.**	**p-value**
Carbapenems	2.11	1.10 -3.97	0.025*

Multivariate analysis was performed using multiple logistic regression analysis.
OR: Odds ratio, CI: Confidence Interval
*p < 0.05 as a statistically significant

Regarding the impact of antimicrobial administration on the acquisition of drug-resistant bacteria, there are some reports that antimicrobial administration within the past 3 months is a risk factor for infection with carbapenem-resistant Enterobacteriaceae[11], that administration of carbapenems increases drug-resistant *Pseudomonas aeruginosa*[12], and that antimicrobial administration increases ESBL-producing bacteria.[13] Antimicrobials are administered to most ICU patients admitted for therapeutic or prophylactic purposes, but increased exposure to antimicrobials can disrupt the nasal flora[8] or intestinal flora.[14] ICU patients are generally in critical condition, often in a relatively immunocompromised state, and constantly at risk of developing opportunistic infections, such as NF-GNR, including *Pseudomonas aeruginosa*, Enterobacteriaceae, and yeast-like fungi, including *Candida* species. Many of these bacteria have acquired multiple drug resistance, often developing difficult-to-treat infectious diseases. Considering that the carriage pressure of *Pseudomonas aeruginosa*, especially drug-resistant strain, and *Candida* species increased with prolonged ICU stays, it would be helpful for both appropriate infectious disease treatment and infection control that being aware of the carriage information of patients admitted to the ICU in advance.

Pseudomonas aeruginosa and *Candida albicans* are opportunistic pathogens frequently co-isolated from critically ill patients in ICU.[15,16] Their interactions can exacerbate patient outcomes through various mechanisms.[17] The antimicrobial usage in ICU patients, especially broad-spectrum antibiotics, can disrupt the normal flora, potentially leading to overgrowth and invasion of opportunistic pathogens due to microbial replacement, as well as promoting the emergence of antimicrobial resistance.[18-23]

The primary purpose of ASC is to grasp the patient's bacterial carriage information; however, an increase in the number of specific bacteria detected does not necessarily mean an increase in the number of patients who develop infectious diseases. Furthermore, delays in appropriate infection control measures can directly spread HAI in a limited space such as an ICU. Regular implementation of ASC can monitor changes in the bacterial flora of ICU patients, allowing for early detection of resistant bacteria and the prompt implementation of additional infection control measures such as contact precautions.[24] ASC needs help with the effort required to implement and its cost-effectiveness. Furthermore, there is still much debate about the usefulness of ASC. It is reported that when ASC was implemented in the ICU, the number of MRSA bacteremia, SSI, and VAP in the entire hospital was reduced[25-27], and that ASC was also useful for controlling carbapenem-resistant Acinetobacter species.[28] In contrast, there is a report that ASC was ineffective in suppressing resistant bacterial infections.[29] For this reason, the Society for Healthcare Epidemiology of America (SHEA) guidelines recommend the active implementation of ASC.[30] In contrast, the Centers for Disease Control and Prevention (CDC) guidelines only recommend ASC in patients at high risk of carriage or in emergencies such as outbreaks[31], showing differing opinions on guidelines. Our study showed that administering broad-spectrum antimicrobials and antifungals affects the increase in *Pseudomonas aeruginosa* and *Candida* species in the nasal cavity. Given the recent global demand for measures against antimicrobial resistance (AMR), the significance of implementing ASC is worth enough from the perspective of appropriate use of antimicrobials. In particular, for patients in ICU, where prophylactic antimicrobials are often administered

perioperatively and broad-spectrum antimicrobials are often administered to critically ill patients, understanding the carriage status of resistant bacteria and other bacteria through ASC would help to control appropriate antimicrobial use.

This study has several limitations. First, it was a retrospective study at a single facility. Second, the selection bias of antimicrobials may have contributed to the study results. In addition, this study cannot state whether nasal colonization with *Pseudomonas aeruginosa* or *Candida* species is directly related to the actual onset of HAI.

In our hospital, the ASC has enabled us to grasp the carriage status of ICU patients, and these data improve staff awareness of infectious diseases. Implementing more appropriate and prompt infection control based on objective data will lead to AMR measures in the ICU as well as throughout the hospital.

Conflict of interest

None of the authors have any conflicts of interest to declare.

Acknowledgements

This work was supported by MHLW Research on Emerging and Re-emerging Infectious Diseases and Immunization Program Grant number JPMH22HA1007.

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