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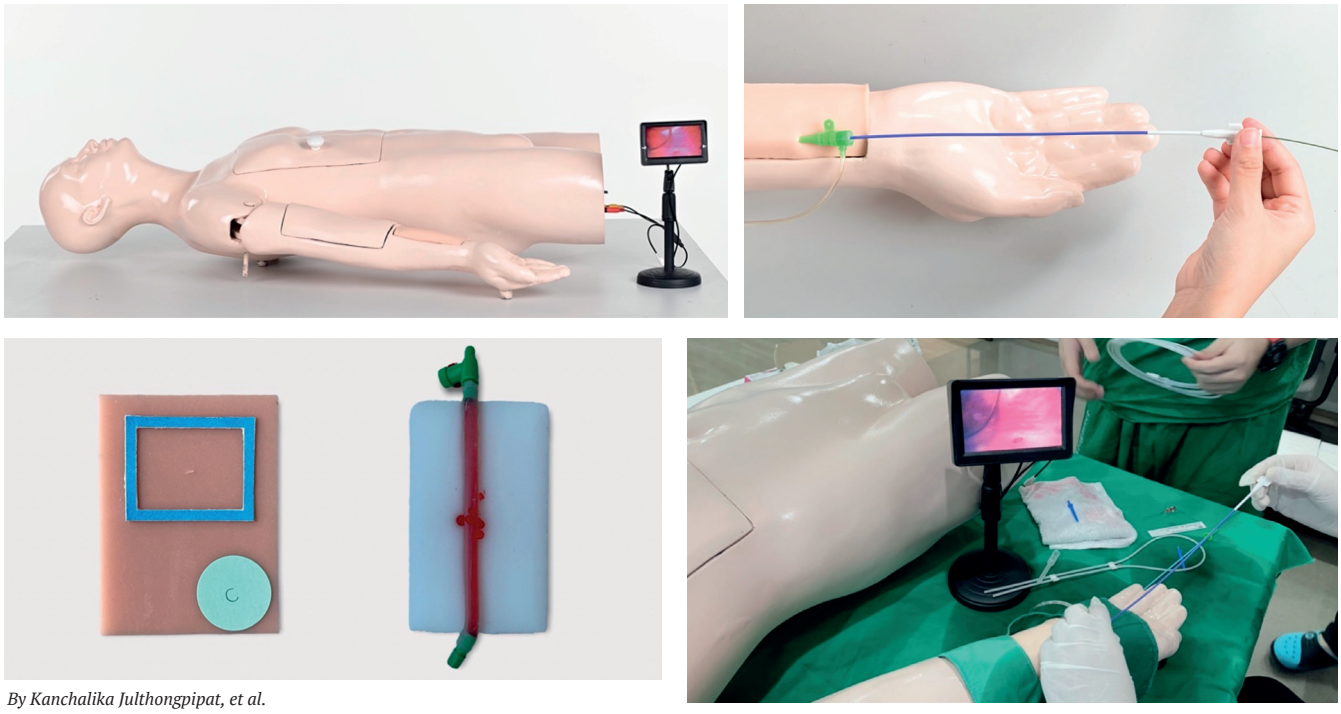

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Factors Predicting Prolonged Postoperative Ileus in Patients Undergoing Major Gastrointestinal Surgery

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ABSTRACT

Objective: This study aimed to investigate the rate of prolonged postoperative ileus (PPOI) and its predictive factors focusing on age, body mass index, smoking status, postoperative mobilization, and preoperative anxiety and depression among patients undergoing major gastrointestinal surgery.

Materials and Methods: This prospective observational study included patients who underwent elective major gastrointestinal surgery in a super tertiary hospital in Thailand. The data were collected using a uniform case record form including the Hospital Anxiety and Depression Scale and Fagerstrom Test for Nicotine Dependence. PPOI was defined using criteria of Vather et al. (2013). Predictive factors for PPOI were determined by multivariate analysis.

Results: A total of 123 patients were enrolled, with an average age of 59.8 ± 12.7 years. The most common indication for surgery was gastrointestinal malignancy (96 patients, 78%), followed by an open approach (75 patients, 61%) and a combined general and epidural anesthesia (58 patients, 47%). Approximately 30% of patients had a history of smoking. Preoperative anxiety and depression were equally found in nine patients (7%). Twenty-seven patients (22%) experienced PPOI. The significant predictive factors of PPOI were having preoperative anxiety (OR = 6.26, 95% CI = 1.22–44.41, $p = 0.046$) and being unable to ambulate on postoperative day 1 (OR = 3.26, 95% CI = 1.25–8.50, $p = 0.015$).

Conclusion: Preoperative anxiety and delayed postoperative ambulation were two predictors for PPOI in this study. Some interventions to reduce preoperative anxiety and encourage early postoperative ambulation should be considered in patients undergoing major elective gastrointestinal surgery.

Keywords: Prolonged postoperative ileus; PPOI; major gastrointestinal surgery (Siriraj Med J 2022; 74: 537-547)

INTRODUCTION

Postoperative ileus (POI), or the temporary dysfunction of bowel motility, is one the most common complications following surgeries involving the gastrointestinal tract. It seems to be an inevitable event since the development of POI is induced in response to the surgery.[1,2] Normally, POI is expected to resolve 2-3 days after surgery as a mechanism of bowel recovery.[3] Therefore, POI that lasts longer than this period is usually considered to be a pathological abnormality, or prolonged POI (PPOI). A wide variety of cutoff times and criteria have been used by studies to determine PPOI; however, 8-32% of patients suffer from PPOI following surgery.[4–13]

PPOI is known to burden both patients and the health care system. Numerous studies have reported that patients with PPOI are prone to develop postoperative

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complications[4,9,13] that can lead to negative outcomes such as a prolonged length of stay[9–11], increased reoperation rate and readmission rate[6,9,11,14], and increased mortality rate.[15] In terms of the health care system, PPOI increases the use of medical resources as well as the economic burden of the treatment.[13]

The literature has shown that several methods can reduce the incidence of PPOI such as laparoscopic surgery,[7,9] chewing gum,[16,17] coffee consumption,[18] postoperative administration of mosapride[19], and early enteral feeding.[20,21] However, some interventions are not applicable in the clinical setting due to several limitations such as patients' condition and health care providers' competency. Moreover, although interventions are applied, the incidence of PPOI remains.

Several factors are associated with the development of PPOI such as age,[6,7] body mass index (BMI),[5,6,22] smoking status,[4,6] time to first ambulation,[12] previous abdominal surgery,[5] and operative time.[6] These factors have been shown to predict PPOI, mostly in retrospective studies of colorectal surgery patients, but conflicting results have been found. Therefore, the factors predicting PPOI in major gastrointestinal surgery remain unclear. In this study, the investigators selected factors based on their potential effect on gastrointestinal motility reported in the literature, including age, BMI, smoking status, preoperative anxiety and depression, and time to first ambulation.

In the literature, advanced age is known to increase the risk, severity, and duration of PPOI due to an imbalance between proinflammatory processes and pro- and anti-inflammatory mechanisms in older adults.[23] In obese patients, it is more difficult to manipulate the fatty tissue surrounding the internal organs, thus resulting in more tissue inflammation and a higher rate of PPOI.[24] Smoking might cause delayed bowel motility as a consequence of nicotine consumption.[25] Anxiety and depression can release catecholamine, which affects the sympathetic innervation of the gastrointestinal tract, thus inhibiting bowel motility.[26,27] Lastly, delayed ambulation might cause a lack of stretch stimulation to the gastrointestinal tract, which is a key factor to enhance intestinal smooth muscle motility and a lack of stimulation to parasympathetic innervation activity, thus decreasing intestinal smooth muscle motility.[28]

Therefore, the purpose of the study was to prospectively determine whether age, BMI, smoking status, time to first ambulation, and preoperative anxiety and depression predict PPOI in major gastrointestinal surgery in Thailand. In particular, the knowledge of such risk factors is crucial, as they could be used to screen patients with a high probability of PPOI, monitor them closely, and manage them suitably to prevent the development of PPOI.

MATERIALS AND METHODS

The sample size was calculated based on a previous study of intraabdominal surgery in which the rate of PPOI was 32.4%.[13] With a power of test of 80% and a significance level of 5% (two-sided test), a sufficient sample size was 123 patients.

Population and samples

The study was conducted in adult patients (aged ≥ 18 years) scheduled for major gastrointestinal surgery in the Faculty of Medicine at Siriraj Hospital, Thailand from February to September 2020. The inclusion criteria were 1) having elective major gastrointestinal surgery; 2) having good consciousness (Glasgow Coma Score ≥ 13); 3) having a Mini-cog score ≥ 3 (patients aged ≥ 60 years); 4) having the ability to walk (with or without assistance); and 5) having the ability to communicate in the Thai language. Patients were excluded from the study if they underwent major gastrointestinal surgery due to trauma or laparoscopic cholecystectomy and appendectomy. Moreover, they were withdrawn from the study if they needed a postoperative ventilator or were discharged before postoperative day 4 and were unable to be contacted by phone.

Instruments

1. Patient record form: The data were collected by interviewing patients and from patients' files. They were divided into three parts. Part 1 (demographic and preoperative data) included sex, age, height, weight, BMI, diagnosis, comorbidity, history of previous abdominal surgery, history of smoking, and method of bowel preparation. Part 2 (intra-operative data) included surgical approach, procedure, anesthetic technique, operative time, and estimated blood loss. Part 3 (postoperative data) included opioid used, time to first enteral feeding, time to first flatus and stool, time to first ambulation, nasogastric tube insertion, complications, reoperation, and length of postoperative stay.

2. Hospital Anxiety and Depression scale (HADS) Thai version.[29] The questionnaire was a self-reported survey comprising 14 items (seven items on the anxiety subscale and seven items on the depression subscale). It was interpreted from its scoring system (0–21 points) as non-case (0–7), doubtful case (8–10), and confirmed case (11–21). The questionnaire was translated into the Thai language and tested with patients with cancer.[29] Both subscales presented good internal consistency, with

Cronbach's alpha coefficients of 0.8551 for the anxiety subscale and 0.8259 for the depression subscale. The questionnaire was used to identify preoperative anxiety and depression in patients undergoing gastrointestinal surgery.[30] Before the study, the questionnaire was tested with 30 patients undergoing major gastrointestinal surgery; the Cronbach's alpha coefficient was 0.82 (unpublished data).

3. The Fagerstrom Test for Nicotine Dependence (FTND) Thai version.[31] The FTND was a self-reported questionnaire comprising six items. The total possible score ranged from 0 to 10. A score of 0–4 was considered as very low to low nicotine dependence, 5 as moderate nicotine dependence, and 6–10 as high to very high nicotine dependence. The intraclass correlation coefficient of the Thai version of the FTND was 0.83 and the Cronbach's alpha was 0.52.[31]

4. Prolonged postoperative ileus: The development of PPOI was determined based on the definition of PPOI by Vather et al.,[32] which included five criteria; 1) nausea or vomiting, 2) the lack of tolerance to an oral diet within 24 hours, 3) the absence of flatus in 24 hours, 4) abdominal distension, and 5) radiologic results of ileus. PPOI was determined if at least two of the criteria were met on the fourth day after surgery. Radiologic evidence of ileus was assessed using the hospital database and the result was confirmed by a physician.

Data collection

Patients who met the inclusion criteria were approached preoperatively on an individual basis and informed about the details of the study together with the right to withdraw at any time. They were ensured that participation in this study would not affect the care and treatment they received. Once patients agreed to participate in the study, they were asked to provide written consent. The data were obtained through questionnaires, interviews, and patients' files. On the first day of admission, patients were asked to complete the HADS questionnaire (15-20 minutes). Patients who had a history of smoking were also asked to complete the FTND questionnaire (5-10 minutes).

The investigators revisited patients on postoperative day 4 between 9 and 10 a.m. to assess whether they had experienced PPOI. However, during the data collection period, if patients needed a ventilator, they were withdrawn from the study. If patients were discharged before postoperative day 4, the investigators called them on postoperative day 4 to assess the development of PPOI.

This prospective observational study was approved by the institutional ethics committee, Faculty of Medicine Siriraj Hospital, Mahidol University (Si 838/2019).

Statistical analysis

The data were analyzed by SPSS version 25 and descriptive statistics were generated. The relationships among the variables were analyzed using a Chi-square test. The powers of the predictive factors were analyzed using binary logistic regression. A p-value of 0.05 was considered to be statistically significant.

RESULTS

During the study period, 132 patients initially met the inclusion criteria. However, nine patients were later excluded from the study: four because their operation was canceled, three because a prolonged postoperative ventilator was used, and two were lost in follow-up. As a result, 123 patients were enrolled, with an average age of 59.8 ± 12.7 years and 52% men. The most common indication for surgery was gastrointestinal malignancy (96 patients, 78%), followed by an open approach (75 patients, 61%) and a combined general and epidural anesthesia (58 patients, 47%). Nearly half (46%) had a history of previous abdominal surgery. The details of patients' demographics and operative characteristics are shown in Table 1.

Approximately 30% of patients had a history of smoking and one-third of them had high to very high nicotine dependence (FTND score 6–10) (Table 2). The same proportion of patients had anxiety and depression preoperatively (7.3% and 7.3%, respectively) (Table 3).

After the operation, around 50% of patients had their first oral feeding on postoperative day 1, first flatus on postoperative day 1, and first stool on postoperative day 2. Up to one-third got out of bed on postoperative day 2. Complications except PPOI were found in 9.8% of patients. Only 1.6% of patients required reoperation. The median postoperative length of stay was five days (IQR 5-7).

As detailed in Table 4, PPOI was observed in 22% of patients undergoing elective major gastrointestinal surgery. In the PPOI group, most patients could not tolerate an oral diet within the first 24 hours (77.8%), had abdominal distension (70.4%), and had nausea/vomiting (66.7%). Only a few patients had an absence of flatus/stool over 24 hours on postoperative day 4 (26%). In particular, PPOI was confirmed by X-ray in 63% of patients with PPOI.

Univariate analysis revealed that having preoperative anxiety (p = 0.025) and starting ambulation on or after postoperative day 2 (p = 0.004) were significantly associated with PPOI (Figs 1 & 2). According to the multivariate analysis, the significant predictive factors of PPOI were having preoperative anxiety (OR = 6.26,

TABLE 1. Patient characteristics and the development of prolonged postoperative ileus (PPOI) (n = 123).

Characteristics	Overall n (%)	PPOI	
		No (n = 96)	Yes (n = 27)
Sex			
Male	64 (52%)	52 (81.3%)	12 (18.7%)
Female	59 (48%)	44 (74.6%)	15 (23.4%)
Age (years)			
Early adulthood (22-40)	14 (11.4%)	11 (78.6%)	3 (21.4%)
Middle adulthood (41-60)	44 (35.8%)	32 (72.7%)	12 (27.3%)
Old age (> 60)	65 (52.8%)	53 (81.5%)	12 (18.5%)
Mean ± SD (years)	59.8±12.7	60.1±12.1	58.9±14.7
BMI (kg/m^2)			
< 18.5	13 (10.6%)	10 (76.9%)	3 (23.1%)
18.5–22.9	53 (43.1%)	38 (71.7%)	15 (28.3%)
23–27.5	35 (28.5%)	30 (85.7%)	5 (14.3%)
> 27.5	22 (17.9%)	18 (81.8%)	4 (18.2%)
Mean ± SD (kg/m^2)	24.34±6.90	24.62±6.82	23.40±7.04
Diagnosis			
Diseases of the liver	25 (20.3%)	24 (96%)	1 (4%)
Diseases of the pancreas	13 (10.6%)	5 (38.5%)	8 (61.5%)
Diseases of the biliary tract and gall bladder	21 (17.1%)	14 (66.7%)	7 (33.3%)
Diseases of the stomach and small intestine	7 (5.7%)	4 (57.1%)	3 (42.9%)
Diseases of the large intestine	46 (37.4%)	40 (87%)	6 (13%)
Morbid obesity	9 (7.3%)	8 (88.9%)	1 (11.1%)
Other	2 (1.6%)	1 (50%)	1 (50%)
Pathology			
Non-cancer	27 (22%)	22 (81.5%)	5 (18.5%)
Cancer	96 (78%)	74 (77.1%)	22 (22.9%)
Comorbidity			
No	33 (26.8%)	22 (66.7%)	11 (33.3%)
Yes	90 (73.2%)	74 (82.2%)	16 (17.8%)
Type of comorbidity (n = 90)			
Diabetes mellitus	27 (30%)	26 (96.3%)	1 (3.7%)
Hypertension	55 (61%)	42 (76.4%)	13 (23.6%)
Dyslipidemia	39 (43.3%)	30 (76.9%)	9 (23.1%)
Coronary artery disease	8 (8.9%)	8 (100%)	0 (0%)
Kidney disease	6 (6.7%)	5 (83.3%)	1 (16.7%)
COPD	2 (2.2%)	2 (100%)	0 (0%)
Other	34 (37.8%)	26 (76.5%)	8 (23.5%)
Previous abdominal surgery			
No	66 (53.7%)	54 (81.8%)	12 (18.2%)
Yes	57 (46.3%)	42 (73.7%)	15 (26.3%)
Smoking status			
Never	84 (68.3%)	65 (77.4%)	19 (22.6%)
Current smoker	12 (9.8%)	11 (91.7%)	1 (8.3%)
Ex-smoker	27 (22%)	20 (74.1%)	7 (25.9%)

TABLE 1. Patient characteristics and the development of prolonged postoperative ileus (PPOI) (n = 123). (Continue)

Characteristics	Overall n (%)	PPOI	
		No (n = 96)	Yes (n = 27)
Bowel preparation			
No	77 (62.6%)	58 (75.3%)	19 (24.7%)
Yes	46 (37.4%)	38 (82.6%)	8 (17.4%)
Type of bowel preparation (n = 46)			
Osmotic agent	37 (80.4%)	30 (81.1%)	7 (18.9%)
Combination of osmotic agent and stimulant laxative	9 (19.6%)	8 (88.9%)	1 (11.1%)
Surgical approach			
Open	75 (61%)	57 (76%)	18 (24%)
Laparoscopy	32 (26%)	31 (96.9%)	1 (3.1%)
Robot-assisted	16 (13%)	8 (50%)	8 (50%)
Procedure			
HPB surgery	45 (36.6%)	38 (84.5%)	7 (15.5%)
Upper GI surgery	15 (12.2%)	12 (80%)	3 (20%)
Colorectal surgery	48 (39%)	42 (87.5%)	6 (12.5%)
Whipple's operation	15 (12.2%)	4 (26.7%)	11 (73.3%)
Anesthesia			
General anesthesia	65 (52.8%)	54 (83.1%)	11 (16.9%)
General anesthesia with epidural anesthesia	58 (47.2%)	42 (72.4%)	16 (27.6%)
Operative time (minutes)			
60-120	2 (1.6%)	2 (100%)	0 (0%)
121-180	14 (11.4%)	13 (92.9%)	1 (7.1%)
181-240	32 (26%)	31 (96.9%)	1 (3.1%)
241-300	27 (22%)	24 (88.9%)	3 (11.1%)
> 300	48 (39%)	26 (54.2%)	22 (45.8%)
Mean±SD (minutes)	317±148	274±113	467±159
Estimated blood loss (ml)			
≤ 500	85 (69.1%)	74 (87.1%)	11 (12.9%)
> 500	38 (30.9%)	22 (57.9%)	16 (42.1%)
Median (IQR)	300 (50–650)	200 (31–500)	550 (300–890)
Postoperative opioid used			
No	9 (7.3%)	9 (100%)	0 (0%)
Yes	114 (92.7%)	87 (76.3%)	27 (23.7%)
Time to the first oral feeding			
Day 0	46 (37.4%)	40 (87%)	6 (13%)
Day 1	67 (54.5%)	50 (74.6%)	17 (25.4%)
Day 2	6 (4.9%)	5 (83.3%)	1 (16.7%)
Day 3	0 (0%)	0 (0%)	0 (0%)
≥ Day 4	4 (3.3%)	1 (25%)	3 (75%)
Time to the first flatus			
Day 1	43 (35%)	39 (90.7%)	4 (9.3%)
Day 2	60 (48.8%)	49 (81.7%)	11 (18.3%)
Day 3	16 (13%)	8 (50%)	8 (50%)
≥ Day 4	4 (33%)	0 (0%)	4 (100%)

TABLE 1. Patient characteristics and the development of prolonged postoperative ileus (PPOI) (n = 123). (Continue)

Characteristics	Overall n (%)	PPOI	
		No (n = 96)	Yes (n = 27)
Time to the first stool			
Day 1	17 (13.8%)	15 (88.2%)	2 (11.8%)
Day 2	44 (35.8%)	38 (86.4%)	6 (13.6%)
Day 3	34 (27.6%)	27 (79.4%)	7 (20.6%)
≥ Day 4	28 (22.8%)	16 (57.1%)	12 (42.9%)
Time to the first ambulation			
Day 1	84 (69.3%)	72 (85.7%)	12 (14.3%)
≥ Day 2	39 (31.7%)	24 (61.5%)	15 (38.5%)
Mean±SD (days)	1.5±0.9	1.3±0.5	2.1±1.4
Nasogastric tube (NGT) insertion			
No NGT insertion	95 (77.2%)	80 (84.3%)	15 (15.8%)
NGT removed by protocol	17 (13.8%)	11 (64.7%)	6 (35.3%)
Delayed NGT removal	9 (7.3%)	4 (44.5%)	5 (55.5%)
NGT reinsertion	2 (1.6%)	1 (50%)	1 (50%)
Complications			
No	111 (90.2%)	94 (84.7%)	17 (15.3%)
Yes	12 (9.8%)	2 (16.7%)	10 (83.3%)
Type of complication (n = 12)			
Intraabdominal collection	4 (33.3%)	3 (75%)	1 (25%)
Surgical site infection	4 (33.3%)	4 (100%)	0 (0%)
Pancreatic fistula	2 (16.7%)	1 (50%)	1 (50%)
Anastomosis leakage	2 (16.7%)	2 (100%)	0 (0%)
Cholangitis	1 (8.3%)	1 (100%)	0 (0%)
Chyle leak	1 (8.3%)	1 (100%)	0 (0%)
Incisional hernia	1 (8.3%)	0 (0%)	1 (100%)
Efferent loop syndrome	1 (8.3%)	0 (0%)	1 (100%)
Septic shock	1 (8.3%)	0 (0%)	1 (100%)
Hospital acquired pneumonia	1 (8.3%)	0 (0%)	1 (100%)
Reoperation			
No	121 (98.4%)	96 (79.3%)	25 (20.7%)
Yes	2 (1.6%)	0 (0%)	2 (100%)
Postoperative length of stay (days)			
2	2 (1.6%)	2 (100%)	0 (0%)
3	20 (16.3%)	20 (100%)	0 (0%)
4	39 (31.7%)	37 (94.9%)	2 (5.1%)
5	21 (17.1%)	21 (100%)	0 (0%)
≥ 6	41 (33.3%)	16 (39%)	25 (61%)
Median (IQR)	5 (4–7)	4 (4–5)	11 (7–22)
Mean ± SD (days)	6.5±5.9	4.5±1.6	13.6±9.5

TABLE 2. Fagerstrom Test for Nicotine Dependence (FTND) and development of prolonged postoperative ileus (PPOI) (n = 39).

FTND score	n (%)	PPOI	
		No (n = 31)	Yes (n = 8)
Very low to low nicotine dependence (0–4)	25 (64.1%)	20 (80%)	5 (20%)
Moderate nicotine dependence (5)	2 (5.1%)	2 (100%)	0 (0%)
High to very high nicotine dependence (6–10)	12 (30.8%)	9 (75%)	3 (25%)
Mean ± SD	1.24±2.27	1.32±2.31	0.93±2.13

TABLE 3. Hospital Anxiety and Depression Scale (HADS) and development of prolonged postoperative ileus (PPOI) (n = 123).

HADS score	n (%)	PPOI	
		No (n = 96)	Yes (n = 27)
Anxiety score			
0-7 (Non-case)	91 (74%)	75 (82.4%)	16 (17.6%)
8-10 (Doubtful case)	23 (18.7%)	17 (73.9%)	6 (26.1%)
11-21 (Confirmed case)	9 (7.3%)	4 (44.4%)	5 (55.6%)
Mean ± SD	5.83±2.94	5.67±2.84	6.41±3.27
Depression score			
0-7 (Non-case)	90 (73.2%)	71 (78.9%)	19 (21.1%)
8-10 (Doubtful case)	24 (19.5%)	18 (75%)	6 (25%)
11-21 (Confirmed case)	9 (7.3%)	7 (77.8%)	2 (22.2%)
Mean ± SD	6.16±2.49	6.16±2.50	6.19±2.50

TABLE 4. Development of prolonged postoperative ileus (PPOI) (n = 123).

Development of PPOI	n	(%)
Development of PPOI		
No (0-1)	96	78
Yes (2-5)	27	22
Diagnosis criteria for PPOI* (n = 27)		
Nausea/vomiting	18	66.7
Lack of tolerance to an oral diet over 24 h	21	77.8
Absence of flatus/stool over 24 h	7	26
Abdominal distension	19	70.4
Radiologic results	17	63

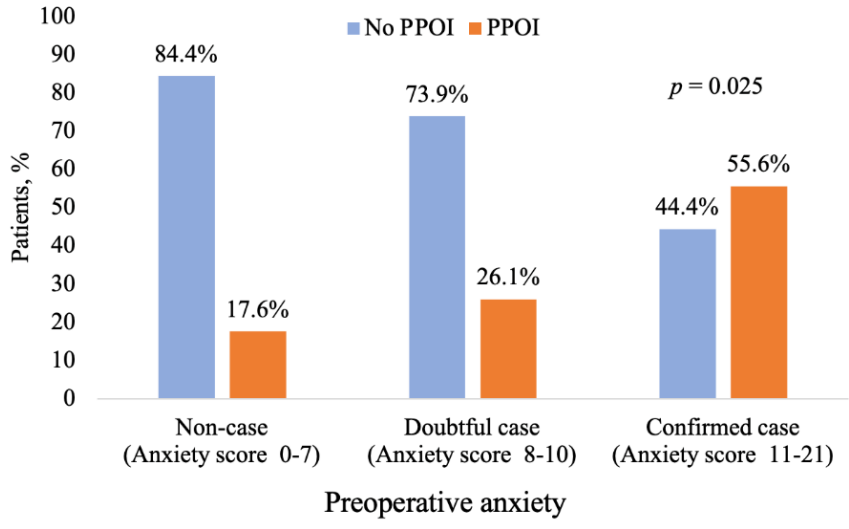

Fig 1. Association between preoperative anxiety and prolonged postoperative ileus.

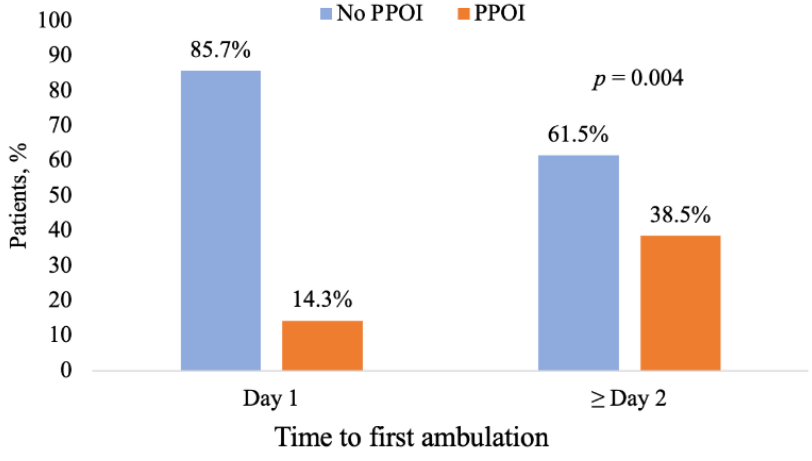

Fig 2. Association between time to first ambulation and prolonged postoperative ileus.

95% CI = 1.218–44.411, p = 0.046) and ambulation on or after postoperative day 2 (OR = 3.26, 95% CI = 1.254–8.501, p = 0.015) (Table 5).

DISCUSSION

This study demonstrated that 22% of patients undergoing major gastrointestinal surgery developed PPOI based on the criteria recommended by Vather et al.,[32] which have been validated and widely used to determine PPOI in several international studies. This study also found that preoperative anxiety and delayed postoperative ambulation were two significant predictors of PPOI. Interestingly, both preoperative anxiety and depression were investigated as psychological distress using HADS in this study, but only anxiety was a significant predictor of PPOI. Several recent studies have addressed the problem of clinical anxiety in elective surgery.[30,34–36] Preoperative anxiety in surgical patients develops for several reasons such as fear of complications, postoperative pain, disability, and death.[34,36] The association between psychological distress and changes in gastrointestinal function has been established in many studies.[37,38] It is known to alter autonomic innervation, specifically stimulated sympathetic innervation, which relaxes the smooth muscle, thus decreasing bowel motility. Another reason might be the release of catecholamine, which is triggered by psychological distress and causes altered mucosal blood flow.[26,27]

Our findings of anxiety (not depression) as a PPOI predictor were consistent with the study by Paine et al.,[39] who examined the relationship between anxiety and depression as well as 24-hour catecholamines in patients with untreated high blood pressure and highlighted that only anxiety is associated with sympathetic innervation

TABLE 5. Univariate and multivariate analysis of the risk factors for prolonged postoperative ileus (PPOI) following major gastrointestinal surgery.

Factors	Crude OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
Age				
Early adulthood[ref]				
Middle adulthood	1.38 (0.33–5.80)	0.664	0.83 (0.15–4.52)	0.832
Old age	0.83 (0.20–3.44)	0.798	0.64 (0.13–3.26)	0.595
BMI (kg/m²)				
< 18.5[ref]				
18.5-22.9	1.32 (0.32–5.45)	0.705	1.19 (0.23–6.11)	0.838
23-27.5	0.56 (0.11–2.75)	0.472	0.57 (0.09–3.55)	0.543
> 27.5	0.74 (0.14–3.99)	0.727	0.73 (0.10–5.39)	0.754
HADS				
Anxiety subscale				
0-7[ref]				
8-10	1.65 (0.56–4.85)	0.359	1.62 (0.48–5.43)	0.439
11-21	5.86 (1.42–24.27)	0.025*	6.26 (1.22–44.41)	0.046*
Depression subscale				
0-7[ref]				
8-10	1.25 (0.43–3.57)	0.683	0.82 (0.24–2.84)	0.759
11-21	1.07 (0.21–5.57)	0.938	0.44 (0.048–3.99)	0.465
FTND score				
0-4[ref]				
5	0.43 (0.15–3.84)	0.989	0.91 (0.02–4.56)	0.899
6-10	0.94 (0.24–3.66)	0.933	0.95 (0.21–4.25)	0.942
First ambulation				
POD 1[ref]				
≥ POD 2	3.75 (1.54–9.12)	0.004*	3.26 (1.25–8.50)	0.015*

*Statistically significant (p-value < 0.05)

Abbreviations: CI; Confidence interval, OR; Odds ratio, BMI; Body mass index, HADS; Hospital Anxiety and Depression Scale, FTND; Fagerstrom Test for Nicotine Dependence, POD; Postoperative day

activation. Therefore, anxiety and depression might affect the gastrointestinal tract through a different mechanism. Another study of 162 surgical patients also confirmed a correlation between preoperative anxiety and delayed gastrointestinal recovery.[40]

The present study also demonstrated that delayed ambulation was significantly associated with PPOI, which concurred with a population-based study in New Zealand in which delayed ambulation was found to influence PPOI following elective colorectal surgery.[12] Meanwhile, early ambulation has been shown to enhance patients' overall recovery, including facilitating bowel motility.[41] Morisawa et al.[28] also found that the bowel sound of critically ill patients is significantly increased after passive exercise. They explained that ambulation can induce gastrointestinal motility by stretching the gastrointestinal tract and stimulating its parasympathetic innervation activity. Moreover, Kumar et al.[42] found that

bowel cleansing is more adequate in patients without ambulation difficulty. Interestingly, as no definition of early ambulation was specified, the results of the study suggested that early ambulation (i.e., postoperative day 1) is recommended, especially to reduce the development of PPOI following major gastrointestinal surgery.

Age was not identified as a significant predictor of PPOI in this study. This concurred with prior studies of colorectal surgery.[5,8,9,12,14,22] BMI was also unable to predict PPOI in this study, again consistent with previous studies.[4,7-9,12,14] Lastly, smoking status was not significantly associated with PPOI in this study, in line with the findings of previous research.[5,9,12]

Although this was a prospective study, some limitations need to be addressed. First, all types of major gastrointestinal surgery were included given that the extent of surgery may vary depending on the nature of the disease (benign versus malignancy) and organ involvement. Second, due to the relatively small number of smokers, the power of smoking could not be determined in this study. Lastly, although PPOI was defined by the international definition proposed by Vather et al.,[32] PPOI may manifest beyond the period of study or after discharge. An extended period of study could provide additional information for calculating the risk factors for the delayed presentation of PPOI.

CONCLUSION

PPOI occurred in 22% of patients following major gastrointestinal surgery. Preoperative anxiety and delayed postoperative ambulation were significant predictors of PPOI. Therefore, it is reasonable that preoperative anxiety should be assessed and treated (if present) under a multidisciplinary team approach. Early ambulation on the first day after the operation is also encouraged alongside effective nursing care and pain control.

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Abbreviations

POI; Postoperative Ileus
PPOI; Prolonged Postoperative Ileus
BMI; Body Mass Index
HADS; Hospital Anxiety and Depression Scale
FTND; The Fagerstrom Test for Nicotine Dependence

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Outcomes of Decompression with Multi-Segment Long Instrumented Fusion in Lumbar Degenerative Disease

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ABSTRACT

Objective: To evaluate the results of DLF in MDLS performed by a single experienced spine surgeon for both radiographic and clinical outcomes.

Materials and Methods: The database of single-institution was retrospectively reviewed. To identify MDLS patients who had undergone DLF (> 2 levels) between 2007 and 2017. Clinical presentation, radiographic measurements, perioperative complications, and postoperative results were analyzed. The Oswestry disability index (ODI) and EuroQol five dimensions' questionnaire (EQ-5D-5L) were used to evaluate the outcomes comparing the preoperative and the most recent postoperative results.

Results: In total, 84 patients (23 males and 61 females) were enrolled, with an average age of 64.4±8.6 (46-81) years old. Among these, 39 patients had fusion to L5 and 45 patients had fusion to the sacrum. Mean operative time was 66.9+23.4 minutes per level (range: 22.2-140) and the average length of stay was 10.7+5.7 days (range: 5-39). The mean estimated blood loss was 290.6+168.5 ml (range: 21.4-666.7). Average follow-up was 50.0+29.8 months (range: 0.5-124). The average preoperative ODI score was 60.6+16.3 (28-97.8) and 24.2+17.3 (0-71.1) postoperatively, while the average preoperative and postoperative EQ-5D-5L scores were 0.161+0.268 and 0.818+0.225, respectively. Both these clinical scores (ODI and EQ-5D-5L) showed a statistically significant improvement ($p < 0.001$). Finally, 13% (11/84) of patients had further surgery.

Conclusion: Decompressive laminectomy and long spinal fusion performed in patients with multi-level deteriorating lumbar spinal stenosis are safe and effective in terms of the patients' quality of life and disability improvement.

Keywords: Lumbar spinal stenosis; multi-level fusion; quality of life; disability; Oswestry disability index (Siriraj Med J 2022; 74: 548-554)

INTRODUCTION

Degenerative disease of the lumbosacral spine can result in back pain, neurogenic claudication due to spinal stenosis, and deformity (both in the coronal and sagittal plane). Surgical treatment for this problem usually consists of a posterior spinal fusion, decompression, and correcting the deformity.[1,2] Long fusion (more than 2 levels) is contemplated for more extensive cases, such as a posterior spinal fusion from L2 to L5 to the sacrum. In patients with deformity, either scoliosis or flat back, an extended fusion up to the thoracic spine or the S2 iliac is required. The surgery is considered quite extensive because of the long operating time, blood loss, and high complication rate.

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This study aimed to evaluate the results of such procedures performed by a single experienced spine surgeon, in terms of both the radiographic and clinical outcomes.

MATERIALS AND METHODS

This study was first obtained by the Siriraj Institutional Review Board approval (Si 031/2012). The study involved a retrospective review of the medical records in the institutional database covering the period between February 2007 to August 2017. The inclusion criteria were patients with the lumbar degenerative disease with spinal stenosis who had undergone long posterior spinal fusion (more than 2 levels) with titanium pedicle screw and rod (Orthipesia®).

Data collection

The data collected were related to the patients' demographic data, including gender, age at surgery, comorbidities, procedure information, implant type, estimated blood loss (EBL), postoperative complications, hospital length of stay (LOS), neurological outcomes, reoperation, time of operation, intraoperative complications, graft type, radiographic findings, and mortality. Radiographic assessments using lumbar lordosis (LL), pelvic incidence (PI), pelvic tilt (PT), sacral slope (SS), and Cobb angle. The Oswestry disability index (ODI)[3] and EuroQol-5 Dimensions Questionnaire (EQ-5D-5)[4] were designed to evaluate the outcomes by comparing preoperative and most current postoperative results to assess the outcomes. The frequencies for categorical and ordinal variables, as well as the averages, standard deviations, and ranges for quantitative data, were determined using descriptive statistics. For continuous variables, the Mann–Whitney U-test was used, and for categorical variables, the Chi-square test was used. For *p*-values of <0.05, statistical significance was assumed, and odds ratios were calculated with the interval of 95 % confidence.

Surgical procedure

In a typical procedure, the patients were situated in a prone post. The paraspinal muscles were then dissected using a midline incision. Pedicular screws were placed on both sides, and decompressive laminectomy was accomplished. Posterolateral fusion was achieved using a local or iliac crest autograft. The operative surgeon determined the number of decompressive levels and fusion levels.

RESULTS

In total, 84 patients were included in this study, comprising 23 males and 61 females. The average age of patients was 64.4±8.6 years old (range: 46–81) and the average BMI was 26.5±4.1 (range: 15.5–37.2). The mean operative time was 66.9±23.4 minutes per level (range: 22.2–140) and the average length of stay was 10.7±5.7 days (range: 5–39). The mean estimated blood loss was 290.6±168.5 ml (range: 21.4–666.7). The average follow-up was 50.0+29.8 months (range: 0.5–124). The average number of fused levels was 4.46+1.85 (ranges 3–9). There were 37, 20, 7, 4, 7, 5, and 4 patients who had fusion involving 3, 4, 5, 6, 7, 8, and 9 levels, respectively, while 39 patients had fusion to L5 and 45 patients had fusion to the sacrum. The patients' demographic data are summarized in Table 1.

The average preoperative LL, PI, PT, and SS were 28.98 ± 17.31(-11–81), 51.98 ± 11.41 (30–76), 27.57 ± 9.36 (3–58), and 24.39 ± 10.24 (-8–49), respectively. The average postoperative LL, PI, PT, and SS were 33.75 ± 13.7 (2–60), 52.49 ± 11.02 (30–77), 25.16 ± 8.05 (0–42), and 27.22 ± 8.44 (5–46), respectively. There were significant differences in LL, PT, SS, and Cobb angle (Table 2). When comparing between patients who were fused at L5 and to the sacrum, there were no significant differences in all parameters. The data are shown in Table 3. In patients with degenerative scoliosis (Cobb's angle of more than 10 degrees), the average Cobb's angle was improved from 23.47 ± 11.20 to 15.11 ± 10.14 degrees.

In terms of clinical outcomes, both the ODI and EQ-5D-5L scores showed a statistically significant improvement ($p < 0.001$). The average preoperative ODI score was 60.6±16.3 (28–97.8) and 24.2±17.3 (0–71.1) postoperatively. The average preoperative and postoperative EQ-5D-5L

TABLE 1. Demographic data of the patients.

Data	Average
Age (year)	64.4 ± 8.6 (46, 81)
BMI (kg/m^2)	26.5 ± 4.1 (15.5, 37.2)
Operative time (minute)	66.9 ± 23.4 (22.2,140)
Length of stay (day)	10.7 ± 5.7 (5, 39)
Estimated blood loss (ml)	290.6 ± 168.5 (21.4, 666.7)
Number of fused levels (level)	4.46+1.85 (3–9)
Follow-up (month)	50 ± 29.8 (0.5, 124)

TABLE 2. Preoperative and postoperative radiographic measurements.

Parameter	Mean Preoperative (degree)	Mean Postoperative (degree)	p-value
LL	28.98 ± 17.31 (-11,81)	33.75 ± 13.7 (2,60)	0.002
PI	51.98 ± 11.41 (30,76)	52.49 ± 11.02 (30,77)	0.410
PT	27.57 ± 9.36 (3,58)	25.16 ± 8.05 (0,42)	0.002
SS	24.39 ± 10.24 (-8,49)	27.22 ± 8.44 (5,46)	0.001
Cobb	15.71 ± 11.95 (1,63)	10.75 ± 9.25 (0,47)	0.000

TABLE 3. Radiographic measurements and outcomes comparing fusion to L5 and fusion to the sacrum.

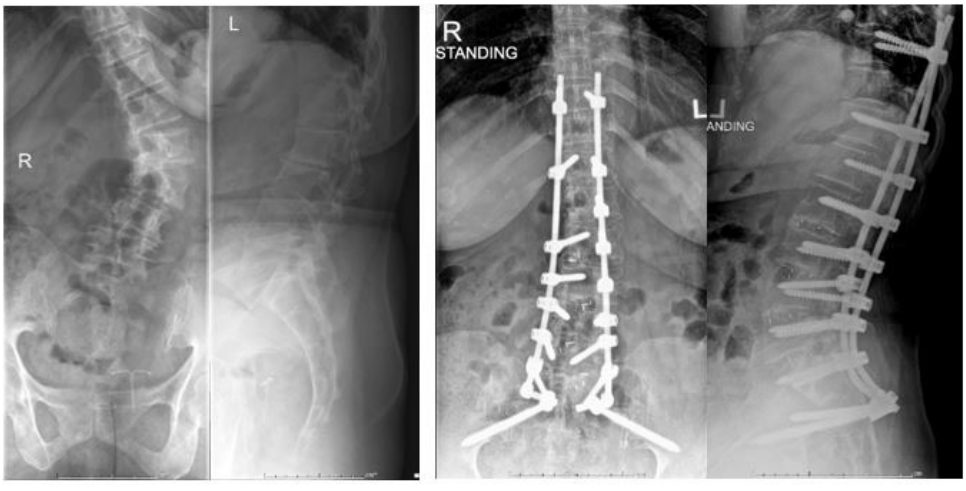
Clinical Measurement	Mean preoperative (degree)	Mean postoperation (degree)	Within group p-value	Between groups p-value
LL				
Fusion to L5 (n = 19)	29.36 ± 19.33	33.21 ± 13.56	0.557	0.962
Fusion to the sacrum (n = 31)	28.64 ± 15.51	34.23 ± 13.97		
PI				
Fusion to L5 (n = 19)	53.28 ± 11.40	53.28 ± 9.97	0.439	0.411
Fusion to the sacrum (n = 31)	50.82 ±11.42	51.80 ± 11.96		
PT				
Fusion to L5 (n = 19)	29.18 ± 10	26.56 ± 7.84	0.797	0.108
Fusion to the sacrum (n = 31)	26.14 ± 8.63	23.91 ± 8.11		
SS				
Fusion to L5 (n = 19)	24.26 ± 8.40	26.72 ± 7.55	0.660	0.758
Fusion to the sacrum (n = 31)	24.50 ± 11.73	27.66 ± 9.21		
Cobb				
Fusion to L5 (n = 19)	15.56 ± 9.45	10.69 ± 6.73	0.916	0.932
Fusion to the sacrum (n = 31)	15.84 ± 13.91	10.80 ± 11.10		
ODI				
Fusion to L5 (n = 19)	60.4 15.7	24.9 17.9	<0.001	0.840
Fusion to the sacrum (n = 31)	60.7 17.0	23.7 17.2	<0.001	
EQ-5D-5L				
Fusion to L5 (n = 19)	0.114 0.236	0.799 0.221	<0.001	0.712
Fusion to the sacrum (n = 31)	0.189 0.285	0.831 0.231	<0.001	

Abbreviations: LL; Lumbar lordosis, PI; Pelvis index, PT; Pelvis tilt, SS; Sacral slope, Cobb; Cobb's angle
ODI; Oswestry Disability Index, EQ-5D-5L; EUROQOL 5 Dimensions 5 Levels

scores were 0.161+0.268 and 0.818+0.225, respectively. The outcomes for patients who had scoliosis before the surgery also improved postoperatively for both scores (ODI from 64.32 ± 15.02 to 23.45 ± 16.78 and EQ-5D-5L from 0.179 ± 0.313 to 0.828 ± 0.221). Additionally, significant clinical improvements were found in both the L5 and sacral fusion groups.

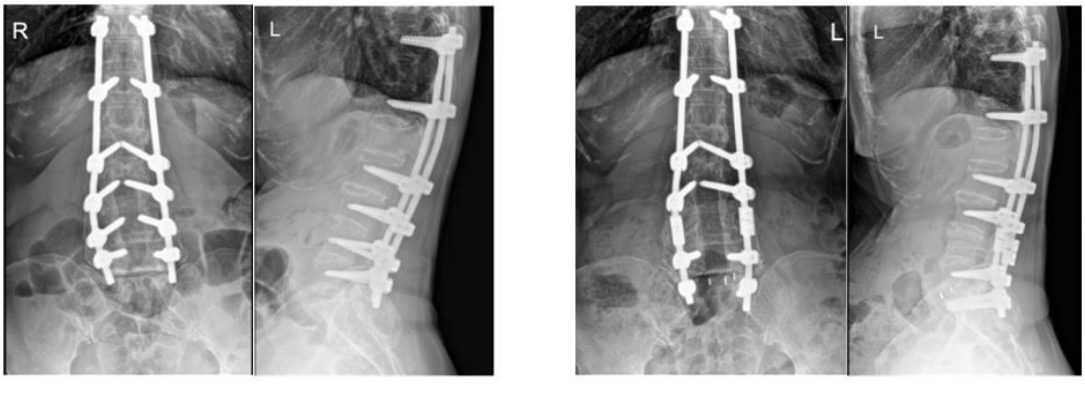
There were cases of 8 dura tears (9.5%). Three patients needed a revision of the pedicle screw due to breaching of the pedicle causing radicular pain. Two patients developed hematoma postoperatively, which required surgical removal at postop days 3 and 5, respectively. Also, massive bleeding due to a segmental artery tear occurred in one patient; while one patient developed a transient ischemic attack; one had superficial wound infection, which required a debridement; one patient had acute cholecystitis; one patient developed left common iliac vein thrombosis; one patient required exploration of the nerve root and removal of the pedicle screw at the L4–5 level, and one patient developed pneumonia postoperatively.

Overall, 11 out of the 84 patients (13.1%) needed further surgery. Three of the 39 patients who had fusion to L5 had extended fusion through the L5–S1 level at 4, 12, and 30 months, respectively, due to a further symptomatic degenerative change in the L5–S1 level. Four of the 45 patients who had fusion through the L5–S1 disc had a revision of the L5–S1 level at 3, 5, 10, and 12 months, respectively, due to displacement of the PEEK in two patients and loosening of the S1 screw in the two other patients. Overall, three patients (3.57%) had extended fusion proximally at 30, 48, and 114 months, respectively. One patient had a proximal junctional fracture and required extended fusion to T4 at 2 months (Figs 2 & 3).



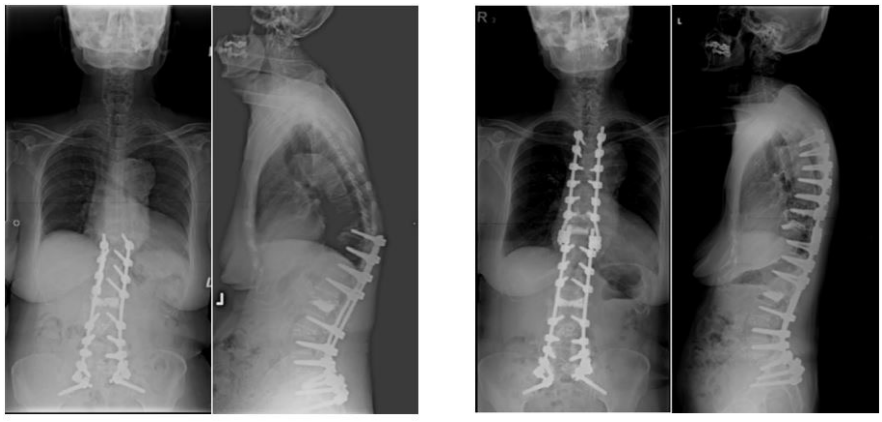
a b

Fig 1. A 61-year-old female underwent a long fusion. The outcomes and Cobb angle were improved postoperatively (ODI from 86.67 to 17.78, EQ5d5L from -0.089 to 1, and Cobb angle from 48 to 17).



a b

Fig 2. A 57-year-old female underwent long fusion from T10 to L5 due to degenerative adult scoliosis with stenosis (a). She had extended fusion to S1 using rod connectors and PEEK rods at nine and a half years (114 months) after the first surgery (b)



a b

Fig 3. A 72-year-old female underwent long fusion from T10 to S2 due to degenerative adult scoliosis with stenosis (a). She had extended fusion to T4 due to proximal junctional fracture at 2 months (b).

DISCUSSION

Degenerative lumbar spinal stenosis (DLS) is a common disease in the elderly. The prevalence of DLS was reported as 5.7–9.3% in a Japanese population and increased with age.[5,6] In terms of radiographic evaluation, the magnetic resonance image (MRI) of the lumbar spine is the gold standard. Srisajjakul and Chawalparit reported the positive predictive values of multiplanar reconstruction computed tomography (MPR-CT) in assessing the central canal stenosis, lateral canal stenosis, and foraminal stenosis were 77.7%, 75% and 50%, respectively. So, the MPR-CT may be considered as a choice of radiographic assessment in DLS patients who had contraindication for MRI.[7]

The current study showed that decompression with multi-segment long instrumented fusion could significantly improve the sagittal profile and clinical score (both ODI and EQ-5D-5L) compared within the preoperative period. These results were related not only to simple degenerative spinal stenosis but also to degenerative scoliosis. Interestingly, the results for a lower instrumented fusion level (L5 vs. the sacrum) did not show a statistically significant difference. Meanwhile, the complication rate was 23.8% (20/84), but surgical-related complications occurred at 17.85%.

The current study concurred with the results of previous research. However, there is some controversy between limited/long fusion and selective/limited decompression. Sun et al. reported the treatment results from 42 patients, Patients with multi-segment lumbar spinal stenosis and single-segment regressive spondylolisthesis were separated into two groups, comprising 22 patients with selective or 20 with multi-segmental compression and fusion. The results showed all the clinical scores (VAS, ODI, and SF-36) at one-year follow-up and three-year follow-up. Both groups were improved considerably compared with their preoperative scores ($p < 0.01$), but no significant differences between the groups were detected at moment in time. However, the multi-segmental compression and fusion group had a longer operative duration and more blood leak. Also, 15 % of the patients in the multi-segmental compression and fusion group had advanced postoperative instability at the adjacent segments above the fused segments at the 3-year follow-up.[1] Lee et al, performed a systematic meta-analysis comparing short limited fusion versus extended fusion with deformity correction in balanced de novo degenerative scoliosis with lumbar spinal stenosis. Six studies involving 362 patients (divided into two groups: 202 short fusions and 160 long fusion) were included in the analysis and the results showed that both groups had a decreased Cobb angle, C7 plumb, and ODI at the final follow-up. Additionally, the lengthy fusion group had showed a significant reduction in Cobb angle and the C7 plumb. However, the short fusion group had lower blood leak (average difference, 739.9 mL) and a shorter operative time (average difference, 68.0 minutes) compared to the long fusion group. Finally, it was concluded that short fusion may be a reasonable option and have a lower risk of curve progression.[8]

Postoperative constipation is a common problem in spine surgery. Siripohn et al conducted a prospective randomized controlled trial comparing only standard nursing care and standard nursing care combined with Thai traditional medicine of abdominal massage (TTMAM group) in patients who underwent lumbar laminectomy. The TTMAM group had a larger percentage of patients who had their first feces between 3 days of surgery and

had reduced abdominal distension on the third day following surgery. Additionally, the TTMAM group's mean patient satisfaction score was greater.[9]

In the present day, the role of minimally invasive surgery has substantially increased. Fan et al. Compared posterior lumbar interbody fusion (PLIF) clinical results versus minimally invasive transforaminal lumbar interbody fusion (MIS-TLIF) in 60 patients who had 3-level degenerative lumbar spinal stenosis. They found no significant variations in back VAS, leg VAS, SF-36, fusion condition, ODI, and difficulties at 12-month follow-up between the two groups ($p > 0.05$). However, the MIS-TLIF group had significantly less blood leak, a shorter hospital stay ($p < 0.05$), and a lower back VAS than those in the PLIF group at 6-month follow-up ($p < 0.05$).[10] Wu et al, showed that a combination of microendoscopic discectomy and minimally invasive transforaminal lumbar interbody fusion in 26 multilevel degenerative lumbar spinal stenosis patients with spondylolisthesis compared with 27 traditional PLIF patients had several advantages, including lower blood loss, less injury to the paraspinal soft tissue, a shorter incision, shorter bed rest time, improved outcomes, and shorter recovery times. However, there were no significant differences between the two groups' operation times or ODI scores.[11] Unfortunately, only open techniques were covered in that study.

Son et al. compared the outcomes between decompression alone versus fusion in elderly patients with two-level or more lumbar spinal stenosis. The results showed that there were no significant differences between the two groups with respect to age, follow-up period, surgical levels, or preoperative condition, though it was found that correction of the lumbar lordosis angle was better in the fusion group at follow-up. However, other clinical outcomes, including VAS, ODI, and Odom's criteria, were not significantly different, but the operation duration. In the decompression-alone group, estimated blood loss and surgical complications were much lower. As a result, the authors concluded that decompressive laminectomy alone was effective in patients with two or more levels of lumbar spinal stenosis who also had a poor general condition or osteoporosis.[12] In Thailand, Keorochana et al. conducted a prospective observational study in 31 degenerative lumbar scoliosis patients with spinal stenosis and reported the effects of decompression and instrumented fusion with a pedicular screw plate system. All of the end indicators, including the pain scales, walking ability, ODI, and Roland Morris score, showed a substantial improvement (p 0.05). Five patients, however, experienced serious problems, with two of them requiring re-operation.[2]

The limitations of the present study include its retrospective nature, a small number of patients, a wide range of follow-up periods, and the use of a single-center database. A large, multi-center, well-controlled study also comparing various fusion techniques could provide additional information about how multi-segment lumbar spinal stenosis is treated in Thailand.

CONCLUSION

This is the first study from Thailand reporting the treatment outcomes of decompression with multi-segment instrumented fusion in lumbar degenerative disease patients. The results showed statistically significant improvements both in the clinical and radiographic results. Additionally, only 13% of patients needed further surgery.

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What is already known on this topic?

The previous study reported good results from decompressive laminectomy and fusion in terms of pain, disability, and quality of life improvements. The use of minimally invasive surgery, including MIS-TLIF or a microendoscopic discectomy combination, also provided non-inferior results with the benefits of lower bleeding and a shorter length of hospital stay.

What this study adds?

The present study showed that decompressive laminectomy and fusion in multi-level spinal stenosis, including degenerative lumbar scoliosis, improved the patients' radiographic outcomes, quality of life, and disability. Also, a lower instrumented level (L5 vertebra or the sacrum) provided good results. However, surgical-related complications (including dural tear, hardware complication, and hematoma) were not uncommon.

Potential conflicts of interest

The authors confirm they have no potential conflicts of interest relevant to this article to declare.

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Clinical Characteristics and Outcome of Bleb-Related Infection in Glaucoma Patients

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ABSTRACT

Objective: To study the clinical characteristics, causative organism, treatment, and clinical outcomes of bleb-related infection.

Materials and Methods: The medical charts of patients who were diagnosed with bleb-related infection, including blebitis and bleb-related endophthalmitis (BRE), from September 2001 to December 2019 at Siriraj Hospital were reviewed. The patients' demographic data, clinical characteristics, microorganisms found, treatment, and clinical outcomes were explored.

Results: We found a total of 42 eyes from 41 patients had been diagnosed with blebitis (11 eyes) and BRE (31 eyes) over the 18-year study period. More than 80% of the patients had experienced pain and redness as the presenting symptoms. The most common bleb characteristic in BRE was purulent bleb (74.2%), while in blebitis it was bleb injection (45.5%). Bleb leakage was documented in 27.3% and 22.6% of patients with blebitis and BRE, respectively. Among the 41 patients, 10 had a history of minor trauma before the onset of infection, such as a rubbed eye, foreign body entering into the eye, water splashed into the eyes, or the eyes had been washed with soap. The yield of vitreous culture in bleb-related endophthalmitis was 48.3%. The most common microorganisms were *Streptococcus spp., Enterococcus spp.,* and *Haemophilus Influenzae*. Generally, the treatment for blebitis at our institute is broad spectrum topical and systemic antibiotics, while intravitreal broad spectrum antibiotics are added to the treatment regimen for BRE patients. For BRE in our cohort, 11 eyes required vitrectomy and 7 eyes had undergone bleb excision. Treatment for blebitis tended to have a good visual outcome, with a stable visual prognosis for 9 out of the 11 eyes diagnosed with blebitis. However, most of the BRE eyes had a worsened visual outcome. *Enterococcus spp.* and *Haemophilus Influenzae* resulted in poor visual outcomes.

Conclusion: The clinical characteristics of bleb-related infection in our patients were pain and redness. One fourth of patients had a history of minor trauma to the eye. To prevent bleb-related infection, the importance of patient education after trabeculectomy should be highlighted. Patients with presenting symptoms and unwanted behavior that could result in bleb infection should be identified and receive treatment alongside education.

Keywords: Bleb infection; blebitis; bleb-related endophthalmitis; clinical; sign; symptom; outcome; predisposing behavior; trauma, patient education (Siriraj Med J 2022; 74: 555-561)

INTRODUCTION

Trabeculectomy is the standard glaucoma treatment and usually reserved as the last resort after intolerable side effects or disease progression become apparent despite the administration of the maximum tolerable topical medications. Glaucoma shunt surgery can cause

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decrease number of endothelial cell count[1] as a long term complication. Trabeculectomy which appear to be safer for endothelial but the most serious complication of trabeculectomy is bleb-related infection. Even though, the incidence of bleb-related infection is rare at only 0.4–0.6%,[1-10] such infection could lead to permanent visual loss and disability. Most studies divide bleb-related infection into two categories depending on the location of the infection: blebitis, which is infection of the bleb and is localized in the anterior segment, and bleb-related endophthalmitis, which is an infection of the bleb and entire globe.

The understanding of the disease presentation, risk factors, causative organism, and outcome of treatment is important to prevent a disastrous outcome of bleb-related infection. The most common causative organisms of bleb-related infection are *Streptococcus spp., Staphylococcus spp., Haemophilus influenzae,* and *Enterococcus spp*.[9,11,12] Blebitis is usually treated with intensive topical antibiotics.[6] The treatment of bleb-related endophthalmitis usually involves intravitreal antibiotics injection, mostly vancomycin and ceftazidime, although pars plana vitrectomy is performed as an alternative approach in some cases.[6,11,12] The visual outcomes of bleb-related endophthalmitis have been reported as: improved (50%), declined (24%), and remained the same (26%).[12] Previous studies found that the risk factors of bleb-related infection were the use of anti-fibrotic agents, such as mitomycin-C or 5-fluorocil[3,9,10,13,14]; inferior bleb approach[10,11,13,15]; fornix-based conjunctival flap[16]; bleb leakage[1,6,11,17,18]; bleb manipulation[11,19], such as bleb needling; and underlying diseases, such as diabetes[20] and blebitis.[20]

Even though trabeculectomy is not an uncommon surgery in the Southeast Asia region in terms of the number of procedures at 122.6-234.0 procedures/1 million persons/year[21], the lack of studies on bleb-related infection is still a problem. Moreover, differences in educational background, personal hygiene, culture, and healthcare accessibility can result in different courses of the disease, as reported in previous studies conducted in other regions. The clinical and microbiological characteristics of bleb-related infection can help guide the ophthalmologist to provide better patient education for improving long-term care regarding post-operative infection prevention and early diagnosis.

Therefore, the primary outcome of this study was to study the symptoms and signs of bleb-related infection. The secondary outcomes were to study the risk factors, causative organism, treatment, and outcome of treatment of bleb-related infection in Siriraj Hospital, Thailand.

MATERIALS AND METHODS

Before we reviewed the medical records, approval for the study was obtained from the Siriraj Institutional Review Board committee, Siriraj Hospital, Mahidol University. We searched the Siriraj Hospital coding database for all patients who were diagnosed with blebitis or bleb endophthalmitis or both. We retrospectively reviewed the records of the patients who were diagnosed with blebitis or bleb-related endophthalmitis or both by diagnosis or by referral to Siriraj Hospital and who were treated at Siriraj Hospital between September 2001 and December 2019. Patients who had a controversial diagnosis of bleb-related infection were excluded from this study. Blebitis was defined as a presumed bleb infection in an eye after filtering surgery when the vitreous was not clinically involved.[2] Bleb-related endophthalmits was defined as an infection invading the anterior chamber and eventually passing into the vitreous.[2] The patients' demographic data, trabeculectomy procedure, co-ocular diseases, diagnosis of glaucoma, management of post-trabeculectomy, signs and symptoms, specimen culture, treatment of bleb-related infection, and outcome of the treatment were reviewed. Stable visual acuity means visual acuity is not deteriorated by more than 2 lines of the Snellen chart or changed from hand movement to the projection of light.

Descriptive statistics were used to evaluate the patients' baseline demographic data, location of the bleb approach, the use of anti-fibrotic agents, type of glaucoma, signs and symptoms of bleb-related infection, the causative microorganism from the culture, type and route of treatment, visual outcome, and post-infection glaucoma status.

RESULTS

After a review of 18 years' medical records, 42 eyes of 41 patients were identified as having been diagnosed with bleb-related infection. The patients' demographic data are shown in Table 1.

The average age at the time of bleb-related infection presentation was 58.5 years old (range 5-87 years old). Seventy-three percent of the patients had bleb endophthalmitis. Nearly a half of patients (47.6%) had underlying primary open angle glaucoma (Table 1).

Symptoms and signs

Blebitis

As shown in Table 2, the most common ocular symptom was redness in 8 eyes (72.7%) follow by pain in 54.5% of patients. All the blebitis patients had conjunctival injection followed by an injected bleb in 45.5% of patients.

TABLE 1. Patients' demographic data.

Demographic data	Number (42 eyes, 41 patients)
Sex	
Male	29
Female	12
Side	
Right	20
Left	22
Age (when BRI; years old)	58.5 (range 5-87)
Diagnosis BRI	
Blebitis	11
Bleb-related endophthalmitis	31
Diagnosis of glaucoma	
POAG	20
PACG	4
Secondary glaucoma	9
JOAG	3

TABLE 2. Signs and symptoms of BRI.

Signs and symptoms	Blebitis (11 eyes)	BRE (31 eyes)
Symptoms		
Pain	6 (54.5%)	28 (90.3%)
Redness	8 (72.7%)	27 (87.1%)
Discharge	6 (54.5%)	20 (64.5%)
Tearing	4 (36.4%)	9 (29%)
Signs		
Conjunctival injection	11 (100%)	31 (100%)
Bleb leakage	3 (27.3%)	7 (22.6%)
Bleb purulent	4 (36.4%)	23 (74.2%)
Bleb injection	5 (45.5%)	7 (22.6%)
Anterior chamber reaction	10 (90.9%)	31 (100%)
Hypopyon	1 (9.1%)	20 (64.5%)
Mild cells	4 (36.4%)	2 (6.45%)
Marked cells	6 (54.5%)	29 (93.6%)
Flare	0	5 (16.1%)
Plasmoid	2 (18.2%)	18 (58.1%)
Fundus examination		
Normal fundus examination	9 (81.8%)	0
Mild anterior vitreous cells	1 (9.1%)	4 (12.9%)
Marked anterior vitreous cells	0	6 (19.3%)
Obscuration due to anterior segment	1 (9.1%)	21 (67.7%)

Almost all the patients (91%) had an anterior chamber reaction, with 54.54% of patients having marked anterior chamber cells. The only patient who didn't have an anterior chamber reaction had purulent bleb. Most of the blebitis cases had normal fundus (81.8%).

Bleb-related endophthalmitis

In contrast to blebitis, 90.3% of patients presented with pain. All the patients had conjunctival injection followed by purulent bleb in 74.2% of patients. Overall, 93% of BRE patients had marked anterior chamber cells with 64.5% of BRE patients having hypopyon. More than half (64.5%) of the patients had obscured fundus (64.5%) (Table 2).

Events

Most the bleb-related infection patients did not have a history of any trauma before. However, two patients had rubbed the affected eye before they were diagnosed as *Enterococcus* BRE. Three patients had a history of a foreign body in the affected eye, such as dust or an insect. The vitreous culture results of these three patients were *Haemophilus, Streptococcus,* and *Staphylococcus*. Two patients had a history of water spreading into the affected eye, with one occurring while cooking and another while praying. One patient had stabbed her finger in the affected eye while washing her face, and another patient had washed the affected eye with soap after eye irritation.

Underlying diseases

The most common underlying diseases found in bleb-related infection were diabetes mellitus in 12/41 patients (29.3%, Blebitis 6/11 BRE 6/31) and hypertension in20/41 patients (48.8%).

History of trabeculectomy procedure

The trabeculectomy site, intraoperative use of anti-fibrotic agents, and types of conjunctival flap and bleb manipulations are shown in Table 3. None of the recorded eyes with trabeculectomy had any other surgery combined or intraoperative complications. Only one eye needed long-term (10 months) topical steroid use after trabeculectomy due to co-morbid ocular disease (chronic anterior uveitis). No long-term topical antibiotics were used.

Bleb manipulations, including 5-fluorouracil subconjunctival injection and bleb needling, were done more than 4 months before the bleb-related infection. There was no history of glue application or tissue plasminogen activator injection before bleb-related infection in our institution.

TABLE 3. History of trabeculectomy and bleb characteristics in BRI patients.

Characteristics	Number (42 eyes)
Trabeculectomy procedure	
Bleb location	
Superior	39
Inferior	3
Intraoperative anti-fibrotic agent used	
Mitomycin-C	26
5-Fluorouracil	1
No use of any anti-fibrotic agent	2
No recorded data	13
Type of conjunctival flap	
Fornix-based conjunctival flap	16
Limbal-based conjunctival flap	11
No recorded data	15
Bleb manipulation	
Needling	6
5-Fluorouracil injection	12
Contact lens wearing	4
Re-suture bleb	4
Autologous blood Injection	2
Bleb revision	1
Bleb characteristic before BRI	
Bleb leakage	11
Cystic bleb	18
Avascular bleb	19
Thin wall bleb	7
Overhanging bleb	1
Flat bleb	2

In BRE patients, 13 eyes had fornix-based conjunctival flap while 7 eyes had limbal-based conjunctival flap with 11 eyes with no record of conjunctival flap type.

The mean duration from trabeculectomy to bleb-related infection was 65.4 months (range 0.33-192.0 months). The characteristics of the blebs before infection are shown in Table 3, with the most common being avascular bleb (55.9%) followed by cystic bleb (52.9%) and bleb leakage (32.4%) There was no characteristics of tense and opaque blebs in the bleb-related infections.

Organisms

Blebitis

The eye discharges from three eyes were sent for culture and the results were *Staphylococcus spp*. for two

eyes and no growth of culture for one eye. The aqueous fluid of three eyes and vitreous of four eyes were sent for analysis and no growth was reported for all (Table 4). There was one eye which both eye discharge and aqueous were sent for culture. The result came back with *Staphylococcus spp*. from eye discharge.

Bleb-related endophthalmitis

In 29 out of a total of 31 eyes with bleb-related endophthalmitis, there was a 48.5% positive rate from the vitreous cultures, in contrast with blebitis, where the yield of the aqueous cultures was zero (Table 4). There were 9 eyes which the specimens were taken from both aqueous and vitreous. Three eyes had positives vitreous culture and 2 eyes had positive result from both specimens with the same causative agents.

Treatment

All the blebitis patients received topical antibiotics. The medication regimens were combined fortified vancomycin and fortified fortum in 5 eyes, combined fortified vancomycin and fortified amikacin in 3 eyes, and combined fortified cefazolin and fortified amikacin, topical levofloxacin, and topical moxifloxacin each in 1 eye. Five of 11 blebitis patients received subconjunctival antibiotics injection. The most common type of antibiotics was subconjunctival vancomycin and ceftazidime injection for 4 cases. Meanwhile intracameral antibiotics injection was applied in only 3 patients. Five patients underwent intravitreal antibiotics injection, comprising combined vancomycin and ceftazidime. Systemic antibiotics were prescribed in 9 patients, including oral levofloxacin in 4 patients, clarithromycin in 2 patients, ofloxacin in 2 patients, and combined intravenous vancomycin and amikacin in 1 patient.

All the bleb-related endophthalmitis patients received topical antibiotics, comprising combined fortified vancomycin and fortified ceftazidime in 22/31 eyes (71%), combined fortified vancomycin and fortified amikacin in 7/31 eyes (22.6%), combined fortified cefazolin and gentamicin in 1 eye, and moxifloxacin in 1 eye. There were 16 and 8 eyes with bleb-related endophthalmitis that received subconjunctival and intracameral antibiotics, respectively. Additionally, 11 eyes underwent vitrectomy. Intravitreal antibiotics were injected in 27 eyes, with the most common being combined vancomycin and ceftazidime in 21 eyes, followed by combined vancomycin and amikacin in 3 eyes, combined vancomycin–ceftazidime–amikacin in 1 eye, combined amikacin and clindamycin in 1 eye, and cefazolin in 1 eye. Most bleb-related endophthalmitis patients were prescribed systemic antibiotics (30 eyes), including combined vancomycin and ceftazidime in 15/30 (50%), and oral levofloxacin in 7/30 (23.33%). Bleb excision was performed in 7 eyes.

TABLE 4. Culture result from various specimens.

Specimen	Blebitis	Bleb-related endophthalmitis
Discharge culture		
Staphylococcus spp.	2	-
No organism found	1	2
Aqueous culture		
Streptococcus spp.	-	1
Haemophilus Influenzae	-	1
Acinatobactor spp.	-	1
No organism found	3	7
Vitreous culture		
Streptococcus spp.	-	5
Enterococcus spp.	-	3
Haemophilus Influenzae	-	3
Staphylococcus spp.	-	1
Morexella spp.	-	1
Candida spp.	-	1
No organism found	4	15

Outcomes

Blebitis

The visual acuity outcome in all eyes after blebitis had subsided was the same as that at baseline before infection. By converting visual acuity to logMAR unit, there was no difference between pre- and post-blebitis. Pre-blebitis was 0.88±1.500 and post blebitis was 0.93±1.497. The glaucoma status was stable in 4 eyes without topical anti-glaucoma being added; however, 3 eyes needed more topical anti-glaucoma after the infection to control the disease, and 4 eyes proceeded toward needing penetrating glaucoma surgery.

Bleb-related endophthalmitis

The visual acuity after the treatment of bleb-related endophthalmitis after 1 year was stable in 9 eyes, but deteriorated by more than 2 lines of the Snellen chart or changed from hand movement to the projection of light in 16 eyes. Using logMAR visual acuity, there was significant deterioration of visual acuity after bleb related endophthalmitis (Mean difference -1.66 ±1.837 95% CI -2.422 to -0.906 p-value = 0.00). Before BRE logMAR visual acuity was 0.57±0.723compare to 2.24±1.888 after the event. The glaucoma status progressed in 27 eyes, including to phthisis bulbi in 13 eyes (42%).

DISCUSSION

This was a study of 42 bleb-related infection cases over 18 years since the first electronic medical records were implemented at the Medical Educational Center, Siriraj Hospital.

Our results showed that pain and redness were the most common ocular symptoms of bleb-related infection. Meanwhile, conjunctival injection was found in all cases of bleb-related infection. Severe anterior chamber reactions (e.g., marked anterior chamber cells, hypopyon, and plasmoid reaction) were reported frequently in bleb-related endophthalmitis. Fundus examination was also hardly performed in bleb-related endophthalmitis. The most common bleb characteristic in bleb-related endophthalmitis was purulent bleb, supported by a "white-on-red" appearance. Most the bleb-related infection patients did not have any history of ocular trauma. However, 24% of the bleb-related infection cases had a history of ocular trauma. There were 2 patients with a history of eye rubbing who were diagnosed with *Enterococcus* bleb-related endophthalmitis. It is known that *Enterococcus spp*. can be found in the gastrointestinal tract of humans, and these endophthalmitis cases indicate low hygiene activities. Patients who had a history of water spreading to the eyes had culture negative organisms or low-virulence organisms (*Morexella spp*.). Nobody had a history of infection from swimming. Some activities reflect insufficient self-care in post-trabeculectomy.

Lehmann reported diabetes was the main risk factor of bleb-related infection.[3] The prevalence of diabetes in patients aged 45-54 years old in Thailand was 9.8%.[4] Diabetes was present in 13% of glaucoma patients.[5] Our first finding reported diabetes in bleb-related infection in 29.3% of cases, which was higher than the prevalence of diabetes in glaucoma patients. Our second finding showed that mitomycin-C was used in 61.9% of procedures. Many studies have found mitomycin-C to be associated with bleb-related infection.[3,6-10] However, recent practice has shifted to mitomycin-C rather than 5-FU. This might result in more percentage of bleb infection in patients who had used mitomycin-C to augment trabeculectomy. Therefore, it was difficult to interpret the association of mitomycin-c use with bleb-related infection. Evidence of chronic blepharitis,[11] the presence of nasolacrimal duct obstruction,[12] and fornix-based conjunctival flap[9] were associated with bleb-related infection. However in our study there was not enough number of eyes and lots of missing data to concluded that fornix-based conjunctival flap is associated with bleb related infection. From previous studies fornix based conjunctival flap as a risk factor of having bleb related infection is still controversy.[13] The number of the eyes having fornix based conjunctival flap is greater which may result from the recent prefer practice of flap type.

In term of other possible risk factors in this study, we did not find any history of other ocular diseases except conjunctivitis in our bleb-related infection study. Keep in mind that this finding is one of the limitations in this study due to retrospective nature which result from the completeness of medical record. Bleb manipulation was reported as a risk factor of bleb-related infection.[12,14] Our findings demonstrated that no bleb-related infection occurred after recent (< 1 month) bleb manipulation. We found that the most common bleb characteristics were cystic and avascular blebs. This could be explained by the thin wall of cystic blebs or micropores, which may allow microorganisms to get into the bleb leading to bleb-related infection.

Previous studies have found *Streptococcus spp*. and *Staphylococcus spp*.[8,12,15,16] to be causes of bleb-related endophthalmitis. The causative organisms of bleb-related endophthalmitis in our study were not only *Streptococcus spp*., but also *Haemophilus Influenzae,* and *Enterococcus faecalis*. In the *Enterococcus* bleb-related endophthalmitis eyes, the patients all had a history of avascular cystic bleb. In the eyes with both aqueous and vitreous specimens

were taken, the positive result have found in vitreous cultures. Therefore it supported that vitreous culture had important role in diagnosing BRE.

Blebitis had a better visual outcome than bleb-related endophthalmitis in our subjects. There was a stable visual outcome in bleb-related endophthalmitis in 9 eyes, with no organism identification in the culture of 6 eyes. The visual outcome in all our *Enterococcus* bleb-related endophthalmitis cases was no light perception, ending up in phthisis bulbi. Therefore, patients with a history of rubbing eyes should be wary of *Enterococcus* bleb-related endophthalmitis. Also, once *Enterococcus* bleb-related endophthalmitis is diagnosed, aggressive and urgent treatment must be delivered and the possible visual outcome should be forewarned to patients. Most importantly, patient education about appropriate self-care after trabeculectomy should be highlighted, particularly regarding eye rubbing and the need for eye protection when doing activities with a high risk of water, dust, or foreign bodies ingress into the eye.

The key limitation of our study to note is the retrospective study design. Bleb-related infection is a rare trabeculectomy complication, thus resulting in a small sample size. However, we reported almost all the aspects and cases of bleb-related infection in our tertiary care medical center in Thailand, which could be beneficial in terms of ensuring patient education to prevent bleb-related endophthalmitis. The symptoms, clinical signs, prognosis, and treatment regimen can present a gross picture for the ophthalmologist to be able to better deal with the bleb-related infection.

CONCLUSION

Bleb-related infection is a rare complication of glaucoma surgery. The clinical characteristics include pain and redness. One fourth of patients in our study had a history of minor trauma to the eye. Appropriate health education for glaucoma patients after trabeculectomy should be provided. Patients with presenting symptoms and unwanted behavior that could result in bleb-related infection should be identified and receive treatment alongside education.

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Long-Term Rehabilitation Outcomes of Neurological Patients: A Multicenter Study

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ABSTRACT

Objective: To evaluate functional outcomes in patients three years after rehabilitation and to identify factors significantly associated with improvement.

Materials and Methods: This prospective cohort study was carried out in nine tertiary centers to compare functional outcomes (Barthel Index; BI) at discharge with scores at three-year follow-up among various diagnoses and types of admissions. Related factors were evaluated for association with improvement in functional score.

Results: Three hundred and eighteen patients (mean age: 54 years; 60% male) were included. More than half of all patients suffered from a spinal cord injury. After three years, 35% of patients were still receiving physical therapy. Only those who were admitted for intensive rehabilitation showed significant improvement after three years. One hundred and ten patients or 35.8% showed significant improvement over time. A univariate analysis showed type of diagnosis, type of admission, onset to admission interval, BI at discharge, and presence of depression and complications at follow-up to be significantly associated with improvements in functional score in the follow-up period. Using a multivariate analysis, only the type of diagnosis, low BI at discharge, and absence of depression and complications at follow-up related to functional improvement.

Conclusion: One-third of patients had sustained functional improvements from rehabilitation three years after discharge. Participants admitted into intensive rehabilitation showed significant improvements in functional scores between discharge and follow-up. TBI diagnosis, low BI at discharge, absence of depression and complications at follow-up related to long-term functional improvement at the three-year mark.

Keywords: Rehabilitation; treatment outcome; inpatients; functions; multicenter study (Siriraj Med J 2022; 74: 562-569)

INTRODUCTION

One of the principal objectives of rehabilitation is to enhance a person's functional status so he/she can participate in normal activities of daily living and social activities, both of which contribute to a better quality of life. Studies from many countries, including a large multicenter trial and three dataset reviews, have shown the effectiveness and efficiency of inpatient rehabilitation to facilitate improvement in or return of patient's normal level of function.[1-4] Several studies have reported factors that predict functional outcomes at discharge from rehabilitation, including age, onset to rehabilitation admission interval (OAI), length of stay (LOS), admission functional status, admission cognitive

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level, diagnosis, intensity of rehabilitation program, and certain characteristics of impairments.[1–3,5,6] In order to achieve the ultimate goal of personal independence and social participation, functional performance needs to be retained long after rehabilitation.

Most studies explore long-term outcomes in stroke patients,[7–9] but some also look at outcomes in patients with a traumatic brain injury.[10–13] As such, functional measurement tools used to evaluate these two patient population groups vary substantially. Only a few studies compare a patient's functional status between rehabilitation discharge and long-term community outcome.[9–11]

Factors reported as being significantly associated with long-term functional outcomes in brain injury patients include age, type of impairment, functional level during admission, functional level at discharge, ability to follow commands before discharge, duration of minimally conscious state, and diagnosis.[8,10,11,13]

In Thailand, there are no rehabilitation units dedicated to a specific type of disorder. All patients in Thailand in need of functional restoration are admitted to general rehabilitation wards located mostly in general hospitals and university hospitals.[14] Data relating to long-term functional outcomes after discharge from inpatient rehabilitation in Thailand is scarce. Accordingly, the aim of this study was to evaluate functional outcomes of patients with neurological conditions after three years of rehabilitation in Thailand, and to identify factors associated with functional outcomes.

MATERIALS AND METHODS

In 2012, two thousand and eighty-one patients from fourteen medical centers participated in a prospective multicenter study to investigate the effectiveness and efficiency of inpatient rehabilitation services in Thailand.[14] Of those, nine centers agreed to participate in this three-year follow-up study of functional outcomes in patients admitted for inpatient rehabilitation. The ethical review committee of each participating center approved this multicenter study (Si: 393/2015). Patients aged above 18 admitted to inpatient rehabilitation facilities in 2012 were included. Those no longer having regular follow-up appointments were contacted via telephone to come back for a checkup. Written informed consent was given by all enrolled study participants.

Demographic and clinical data included age, gender, onset-to-admission interval (OAI), length of stay, marital status, diagnosis, requirements for caregiver, discharge location, and type of admission. Participants were examined by a rehabilitation physician for functional status using the Barthel Index (BI)[15] at their three-year follow-up visit. The BI ranges from 0-20, with a higher score indicating better functional status. The BI score at three-year follow-up was compared to the BI score at discharge among various diagnoses and types of admission. In addition, the number (%) of patients with different levels of disability severity was analyzed for association between discharge and three-year follow-up.

The type of admission was classified as either intensive or non-intensive rehabilitation. Patients admitted in the intensive program included people who could tolerate rehabilitation at least three hours per day, five days per week. Patients admitted in the non-intensive program satisfied one or more of the following criteria[14]: 1) patient could tolerate rehabilitation less than two hours per day; 2) admitted for investigation; 3) admitted so that their caregiver could receive training; and/or, 4) admitted to be treated for complications. Readmission after discharge from inpatient rehabilitation wards was also recorded. Complications were recorded as none or having complications, including pain, spasticity, shoulder subluxation, joint contracture, pressure ulcer, pneumonia, incontinence, dysphagia, and depression. Depression was evaluated using the Patient Health Questionnaire (PHQ-9) with a score greater than nine indicating depression.[16]

Statistical analysis

Demographic and clinical data was analyzed descriptively. A paired t-test was used to analyze changes in BI scores at discharge (BI_{DC}) and at three-year follow-up (BI_{FU}) among various diagnoses and types of admission. The McNemar's test was used to compare severity of disabilities at discharge and three-year follow-up. Factors related to changes in functional score were analyzed using the Student's t-test and one-way analysis of variance (ANOVA) for categorical data. The Pearson's and Spearman's rank correlation coefficient tests were applied for continuous data. All factors with a p-value of less than 0.10 in univariate analysis was included in a multiple linear regression analysis. A p-value of less than 0.05 was regarded as being statistically significant. All statistical analyses were performed using SPSS Statistics version 18 (SPSS, Inc., Chicago, IL, USA).

RESULTS

Among the 1,431 patients admitted to nine hospitals in Thailand in 2012, we were able to track 628 (43.89%). Of those, 109 had died, 201 declined to participate, and the remaining 318 patients were included in this study (Fig 1). Ninety-four patients were readmitted to the hospital after discharge in the three-year follow-up period, and approximately half of those had more than

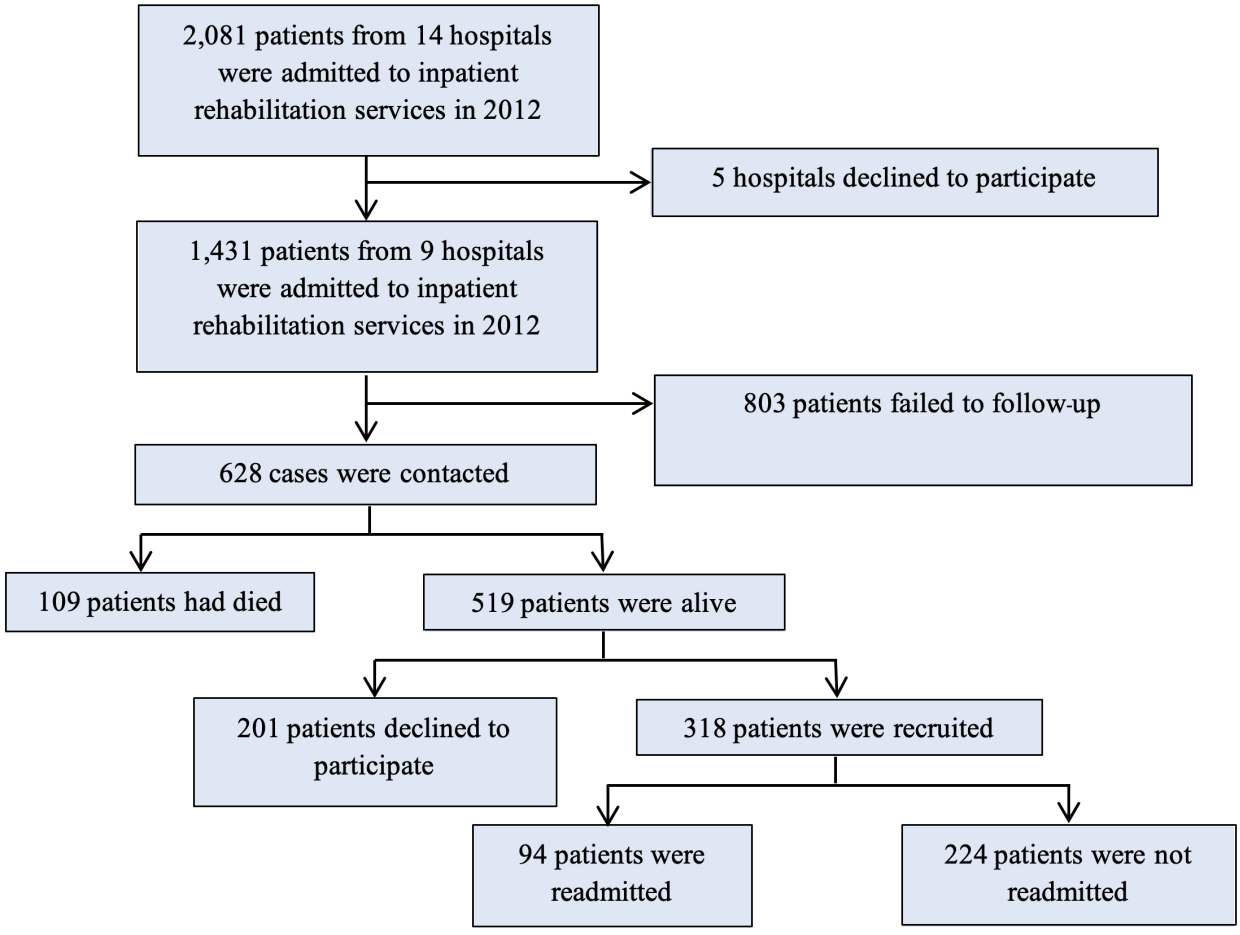

Fig 1. Flow diagram of study enrollment process

one admission due to complications. The majority of complications were urinary tract infection (UTI) and pressure ulcer, and a small number of patients had pneumonia, deconditioning, or recurrent stroke.

The average age of patients was 54, with 60% being male. More than half of all patients suffered from spinal cord injuries (SCI). Approximately 80% of study patients also required assistance from a caregiver. More than half (53.6%) of patients were admitted at some point in time for intensive rehabilitation (Table 1).

The Barthel Index (BI) compared type of diagnosis and type of admission at discharge and the follow-up period as shown in Table 2. The changes in BI score at follow-up and discharge revealed statistically significant improvements regarding stroke, SCI, and traumatic brain injury (TBI). Patients with TBI showed the greatest increase in functional score. In addition, only those who were admitted for intensive rehabilitation showed significant improvements at the three-year follow-up.

Association between functional score (BI) at discharge and at the three-year follow-up by severity of disability is shown in Table 3. Almost half of all patients (46.3%) remained at the same level of ability, while 110 (35.8%) showed signs of improvement over time. Only 55 patients (17.9%) had functional deterioration at the three-year follow-up mark.

A univariate analysis revealed type of diagnosis (p=0.039), type of admission (p=0.025), onset-to-admission interval (OAI) (p=0.034), BI at discharge (p<0.001), presence of depression at follow-up (p=0.015), and presence of complications at follow-up periods (p=0.059) to be significantly associated with improvement in functional score at follow-up. A multivariate analysis identified four factors that were either negatively or positively associated with improvements in functional score at three-year follow-up. TBI diagnosis positively correlated with improvements in functional score at follow-up (p=0.048). A low BI score at discharge (p<0.001), presence of complications at follow-up (p=0.011), and presence of depression at follow-up (p=0.023) all negatively correlated with improvements in functional score at follow-up (Table 4).

TABLE 1. Demographics and clinical characteristics of study participants.

Characteristics	(N=318)
Age (years), mean ± SD	54.0 ± 18.5
Male gender, n (%)	191 (60.1%)
Onset-to-admission interval (months), median (min, max)	5.00 (0.03, 312.00)
Length of stay (days), median (min, max)	20 (1, 236)
Marital status: married (n=314), n (%)	98 (31.2%)
Need caregiver, n (%)	252 (79.2%)
Discharge location: home (n=309), n (%)	259 (83.8%)
Diagnosis (n=315), n (%)	
Stroke	80 (25.4%)
Spinal cord injury	168 (53.3%)
Traumatic brain injury	11 (3.5%)
Others	56 (17.8%)
Type of admission (n=304), n (%)	
Intensive rehabilitation	163 (53.6%)
Less-intensive rehabilitation	24 (7.9%)
Investigation	73 (24.0%)
Caregiver training	9 (3.0%)
Treatment of complications	11 (3.6%)
Others	24 (7.9%)

TABLE 2. Barthel Index compared between discharge and follow-up for type of diagnosis and type of admission.

Variables	n	BI discharge (mean ± SD)	BI follow-up (mean ± SD)	Change score (95% CI)	*P*-value[#]
Diagnosis					
Stroke	77	12.6 ± 4.8	14.0 ± 6.7	1.4 (0.4, 2.5)	***0.007***
SCI	163	11.6 ± 5.4	12.4 ± 5.6	0.8 (0.2, 1.5)	***0.007***
TBI	10	8.4 ± 5.6	13.1 ± 7.5	4.7 (2.5, 6.9)	***0.001***
Others	54	13.9 ± 5.3	14.6 ± 6.3	0.7 (-0.4, 1.9)	0.203
Type of admission					
Intensive rehab	163	12.1 ± 4.9	13.7 ± 6.4	1.6 (0.9, 2.3)	***<0.001***
Less-intensive rehab	24	8.0 ± 5.0	8.8 ± 5.7	0.8 (-1.1, 2.8)	0.383
Investigation	73	13.4 ± 4.8	13.7 ± 4.8	0.3 (-0.5, 1.1)	0.428
Caregiver training	9	3.1 ± 4.0	3.8 ± 5.3	0.7 (-3.6, 4.9)	0.728
Treat complications	11	13.6 ± 2.7	14.8 ± 4.3	1.2 (-1.4, 3.9)	0.308
Others	24	16.0 ± 4.8	15.9 ± 5.0	-0.1 (-0.9, 0.8)	0.919

A *p*-value <0.05 indicates statistical significance; [#]Paired t-test

Abbreviations: BI; Barthel Index, SCI; spinal cord injury, TBI; Traumatic brain injury

TABLE 3. Association between functional score at discharge and functional score at the three-year follow-up by severity of disability (N=307).

Severity of functional score (BI) at discharge n (%)	Severity of functional score (BI) at 3-year follow-up, n (%)				
	Very severely disabled (BI: 0-4)	Severely disabled (BI: 5-9)	Moderately disabled (BI: 10-14)	Mildly disabled (BI: 15-19)	Independent (BI: 20)
Very severely disabled (BI: 0-4)	25 (8.1%)	9 (2.9%)	2 (0.7%)	1 (0.3%)	0 (0.0%)
Severely disabled (BI: 5-9)	9 (2.9%)	13 (4.2%)	10 (3.3%)	14 (4.6%)	1 (0.3%)
Moderately disabled (BI: 10-14)	6 (2.0%)	13 (4.2%)	51 (16.6%)	33 (10.7%)	13 (4.2%)
Mild disabled (BI: 15-19)	0 (0.0%)	2 (0.7%)	17 (5.5%)	37 (12.1%)	27 (8.8%)
Independent (BI: 20)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8 (2.6%)	16 (5.2%)

McNemar's test: p-value<0.001
Abbreviation: BI, Barthel Index

DISCUSSION

Three years after discharge from rehabilitation, our study found that one-third of all patients had sustained functional improvement, while almost half remained at the same level of disability, and one-fifth had functional deterioration. Wade and Hewer reported the outcomes at six months post-stroke and found that 65.7% of patients saw an improvement while the condition of 6.2% declined.[17] Our results are in contrast against those in a study by Kuptniratsaikul, *et al.* who reported that 51.5% of patients had improved functional level while 12.8% saw a decline one year post-stroke.[18] These studies confirm the natural course of functional recovery in which significant improvement occurs within 6-12 months, with a subsequent observed tendency of the patient's functional level to stabilize or decline thereafter. It is important to emphasize that in addition to our study which enrolled stroke patients (25.4%), we also enrolled patients with SCI (53.3%) and TBI (3.5%). More than 80% of participants were admitted with sub-acute neurological conditions and therefore, the course of recovery discussed here is probably applicable.

In addition to observed significant improvements in functional score at three-year follow-up, patients admitted for intensive rehabilitation showed apparent sustainability of functional status, which is consistent with previous findings.[14,19,20] Pattanasuwanna and Kuptniratsaikul performed a retrospective study in stroke patients admitted between 2010-2014 to investigate five-year outcomes,[19] and Rinkaewkan and Kuptniratsaikul performed a retrospective study in patients admitted for SCI rehabilitation between 2006-2010.[20] Those studies revealed that stroke and SCI patients admitted for intensive rehabilitation had better functional improvements (change in BI score) than groups admitted for non-intensive rehabilitation. These differences may be due to the fact that patients admitted for intensive therapy were deemed able to tolerate a three-hour per day program. Taken together, these findings also confirm previously reported findings that intensive rehabilitation is effective for improving functional status in any disease.[14]

Regarding factors that significantly correlate with an improved Barthel Index score over time, our study found four. A multivariate analysis showed that diagnosis of TBI positively associated with improved BI at follow-up, low BI at discharge, and that presence of depression at follow-up, and presence of complications at follow-up negatively associated with improved BI. This result aligns

TABLE 4. Univariate and multivariate analysis for factors significantly associated with changes in Barthel Index score (BI_{FU} - BI_{DC}).

Factors	n	Univariate analysis		Multivariate analysis		
		Change in BI score	*P*-value	b	SE (b)	*P*-value
Age (years)	305	r = -0.064	0.268	-	-	-
Gender			0.838	-	-	-
Male	184	1.1 ± 4.2				
Female	123	1.2 ± 4.5				
Marital status			0.659	-	-	-
Single	95	1.1 ± 4.2				
Married	160	1.2 ± 4.5				
Divorced	48	0.6 ± 5.3				
Diagnosis			***0.039***			
Others	54	0.8 ± 4.3				
Stroke	77	1.5 ± 4.7		0.324	0.751	0.667
Spinal cord injury	163	0.9 ± 4.1		0.052	0.677	0.939
Traumatic brain injury	10	4.7 ± 3.0		3.052	1.558	***0.048***
Discharge location			0.639	-	-	-
Home	273	1.2 ± 4.4				
Nursing home/shelter	26	0.8 ± 3.5				
Type of admission			***0.025***			
Non-intensive	141	0.5 ± 3.8				
Intensive	163	1.6 ± 4.7		0.430	0.565	0.447
OAI	294	r = -0.123	***0.034***	-0.009	0.005	0.078
LOS	307	r = 0.067	0.243	-	-	-
BI at discharge	307	r = -0.194	***0.001***	-0.207	0.047	***<0.001***
Readmission during 3 years			0.143	-	-	-
Yes	90	0.5 ± 4.8				
No	217	1.3 ± 4.1				
Presence of $depression_{FU}$			***0.015***			
No	228	1.5 ± 4.3				
Yes	70	0.1 ± 3.7		-1.325	0.579	***0.023***
Presence of $complications_{FU}$			***0.059***			
No	49	2.2 ± 4.0				
Yes (any complications)	258	0.9 ± 4.4		-1.781	0.694	***0.011***

P-values <0.1 and <0.05 indicate statistical significance in univariate and multivariate analysis, respectively

Abbreviations: SE; standard error, OAI; onset-to-admission interval, LOS; length of stay, BI; Barthel Index, FU; follow up, DC; discharge

with those from a study by Katz, *et al.*,[11] which suggests that TBI patients have a better chance of recovery than non-TBI patients. The functional status at admission or discharge from rehabilitation has also been demonstrated to be an indicator of level of ability or disability in the long-term in many studies.[8,10,11,21-24] As there is a variety of measurement tools being used, it is difficult to directly compare the results reported among studies.

Cognitive function is one factor that can predict functional outcomes at discharge from rehabilitation. Rehabilitation doctors do TMSE for cognitive function screening as a routine during inpatient service. In addition, only stroke patients with good potential are suitable for receiving rehabilitation program. That means they should have good co-operation, be able to follow command and retain 24-hour memory. These can represent fair to good cognitive function. Depression, another important factor, is one of our obstacles to rehabilitation program. It could affect co-operation and final outcomes. Every inpatient in rehabilitation ward was also screened for depression as a routine procedure. Other studies reported the fact that depression is an independent indicator of poor functional status in the long-run.[18,25,26]

Concerning complications, Rinkaewkan and Kuptniratsaikul conducted a retrospective study in 201 SCI patients admitted to rehabilitation wards between 2006-2010. They found an absence of comorbidity and the ability to undertake an intensive rehabilitation program to be associated with increased functional score after rehabilitation.[20]

Discharge to home is one of the final outcomes which represents successful rehabilitation program. Our country could not provide skilled nursing facilities like other western countries, so one of our goals is patients being discharged to home after receiving full rehabilitation program. In addition, some patients still had disabilities that needed help at discharge. Caregiver was a key person to help patients complete those functions at home. However, to minimize the burden of caregiver, they had to learn how to help patients independent before being discharged. Rehabilitation team should also concern burden of caregiver as well as patients' quality of life.[27,28]

This study had some limitations such as the fact that only 318 of 1,431 patients from nine medical centers could be recruited, which is a relatively small study population. In contrast, a study conducted in the United States was able to collect rehabilitation outcome data of more than 200,000 patients in more than 300 rehabilitation centers over an eight-year period.[29,30] In countries lacking long-term data collection systems like Thailand, the analyzed information is from a relatively small number of patient records and from postal surveys, which are associated with low response rates.[7,11–13] Moreover, it may be inferred that data from these two methods would probably reflect more well-disciplined patients that maintained contact with health care providers. It is also, therefore, possible that the health status and functional level of participants included in the present study would be better than the health status and functional level of participants who failed to follow-up. It should also be noted that funding limitations prevented the research team from visiting patients at their homes. Further complicating the patient recruitment process was that some patients lived in rural areas which are inconveniently far from a rehabilitation center, so many declined to participate. A further study with more participants would be helpful in confirming long-term functional outcome findings reported in this study.

CONCLUSION

More than one-third of patients had sustained improvements in functional level three years after discharge from inpatient rehabilitation. Participants admitted for intensive rehabilitation showed significant improvements in functional scores between discharge and three-year follow-up. A multivariate analysis revealed TBI diagnosis to be positively correlated, and low BI at discharge, presence of depression, and presence of complications at follow-up to be negatively correlated with long-term functional improvements.

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Conflicts of interest: All authors declare no personal or professional conflicts of interest regarding any aspect of this study.

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Development of Simulation Model for Transradial Catheterization Practice for Physicians

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ABSTRACT

Objective: This study tested different types of silicone rubber material to assess the most durable with properties that best simulated the human skin and vascular. The optimal materials were used to produce a transradial catheterization simulation model to train medical practitioners and tested the improvement of the training with a medical simulation model.

Materials and Methods: Three types of silicone rubber were tested for their suitability as artificial skin and vascular for transradial catheterization simulation model. Eighteen fellowship physicians assessed the simulator's operational effectiveness and recorded their satisfaction with the training.

Results: Silicone rubbers were tested for realism and capability for repetitive training. Silicone rubber RTV-01 was the most durable for simulating the artificial skin, while silicone rubber RTV-03 was the most durable for simulating the artificial vascular with statistically significant results recorded by Kaplan-Meier analysis ($P < 0.1$). Satisfaction assessment results of the 18 participants using a Likert scale (5 points) returned total average scores of model's efficacies as 4.41 and total average scores of model's usefulness as 4.59.

Conclusion: The materials were used for transradial catheterization simulation to enhance fellowship trainees' learning efficiency through practice. The fellowship trainees became familiar with the equipment, gained a higher completion rate, and increased confidence in treatment planning.

Keywords: Transradial catheterization; medical simulation model; silicone rubber (Siriraj Med J 2022; 74: 570-574)

INTRODUCTION

Coronary artery disease (CAD) or ischemic heart disease (IHD) is the leading annual cause of death worldwide.[1] Coronary angiography (CAG) is the gold standard procedure used to diagnose blocked areas in coronary arteries. Vascular access for coronary angiography can be performed via the femoral artery at the groin or via the radial artery at the wrist. A transradial catheterization procedure is safer than a femoral artery approach. The patient recovers faster, with a reduced risk of complications after the procedure[2-4]; however, vascular system access via the radial artery requires skillful catheter insertion into a smaller vessel than the femoral artery.

Currently, diagnostic cardiac catheter training is performed directly on patients under the supervision of a senior fellowship or staff physician. The Siriraj Hospital Faculty of Medicine has modernized instruction methods that now promote outcome-based education[5], with learning and teaching styles focusing more on treatment outcomes. Practical approaches are now encouraged to supplement

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lectures.[6] However, patient numbers are often insufficient to allow students adequate medical practice for many of the procedures, and as a result, some students lack confidence when treating patients. Practicing simulation techniques allows the students to gain experience before performing in real operation settings. Simulated training methods improve confidence and proficiency, allowing detailed preparation of each treatment step.[7-15] Due to the recent coronavirus COVID-19 pandemic, medical institutions actively adapt their teaching approaches to prevent infection. The medical model is the best learning tool to enhance medical practitioners' skills to the fullest potential without concerns.[16] Practitioners can practice individually with the model to compensate for practicing with patients. Moreover, the medical model can reduce instructional costs by repetitively use and being able to create the format related to instructors' guidance.[17]

A previous study demonstrated higher operation success rates after simulation training.[18] A VIST-C endovascular simulator is currently used in some cardiac catheterization practices. This high-precision device assists in practicing procedures as primarily visual responses in functional systems covering a wide range of vascular pathologies and standardized clinical complication situations in examination training. Using simulation scenarios significantly improved cardiac catheterization skills through training and reduced real device training process time in the Cath Lab.[19] A TSP simulation model was previously developed to train interventional cardiologists in cardiac catheterization. X-ray image simulation (fluoroscopy) and a wide variety of vascular pathology using 3D programs have also been used to assist real learning. Rating test results of the interventional cardiologists indicated the suitability of using simulation models; however, these results were not extensively tested because only interventional cardiologists were enrolled in the study.[20] Commercially available catheterization simulators include the ANGIO Mentor Suite produced by Simbionix Simulators, with simulation training of basic skills in Electrophysiology. However, this test does not cover the actual circulatory vascular system, while the simulation model looks like a box, lacks realism and does not match actual operation procedures. Simulation models for transradial catheterization operations are limited by their high cost.[21] Previous studies investigated the use of simulation models to practice complex procedures, with results showing reduced procedure time, fluoroscopy time and contrast media volume.[22] Studies on teamwork or computational safety of skilled participants are limited compared to assessments on simulation model realism and functionality.[23] To overcome these limitations, a half-body human-size simulation model was developed for trainee physicians to practice transradial catheterization via the radial artery in the wrist. The study subjects were fellowship students in the Interventional Cardiology Program.

MATERIALS AND METHODS

Artificial skin and radial artery selection steps following: 1) The three silicone rubber specimens that were all readily accessible on the market were analyzed. Each of the three types of silicone rubber had a Shore hardness value similar to human skin and the radial artery. Human skin has a Shore A hardness grade of 10 to 20, while the radial artery has a Shore A hardness grade of 37 to 39.[24-25] 2) The silicone rubber specimens were repeatedly punctured with an actual needle 20Gx$^{1}/_{4}$ 1 in the exact location until a defect occurred as needle marks and tearing of the material. Survival analysis followed the Kaplan-Meier method. 3) Three experts evaluated the material qualities used to create the model. A questionnaire was used to collect information from the interventional cardiologist participants for model reliability, including Intraclass Correlation Coefficient (ICC) statistic, and 10 points were evaluated. 4) This experimental research measured the efficiency and satisfaction of the research population as 18 fellowship students from the Interventional Cardiology Program who had previous and no previous operation experience.

A half-body human-size simulation model, 108 cm high and 53 cm wide was created to improve transradial catheterization practice skills for the trainee physicians. Suitable materials were used to replicate the skin and artificial blood vessels. Three cardiologists conducted the testing procedure on 18 fellowship physicians in interventional cardiology studying the sub-discipline of cardiovascular disease treatment. Training using the simulation model consisted of two steps 1. vascular access and 2. catheter insertion to the coronary artery. (Figs 1 & 2)

Fig 1. Transradial catheterization simulation model

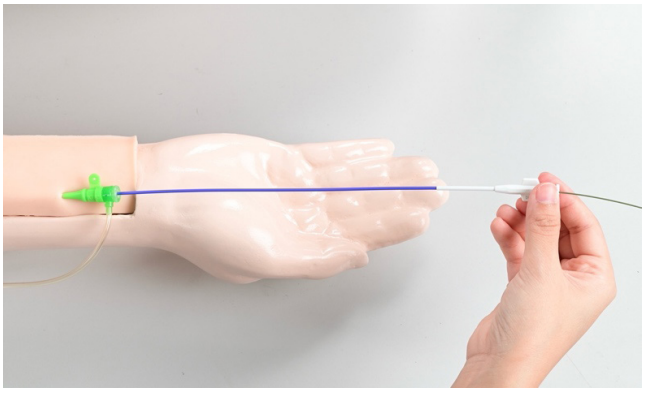

Fig 2. Coronary artery angiography training using the simulation model

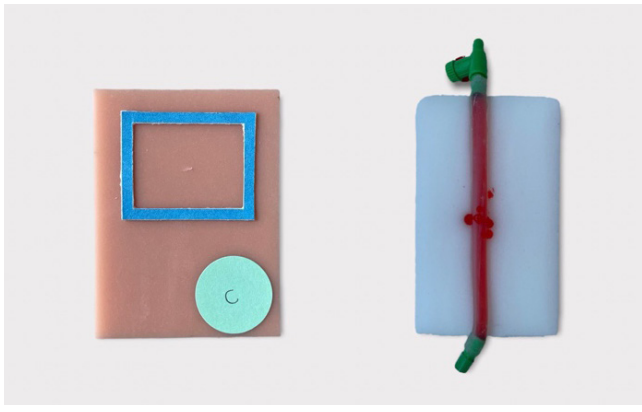

Fig 3. Survival analysis showing the needle punctures until the silicone rubbers were damaged

RESULTS

Silicone rubber durability test

Silicone rubber durability was tested by determining the number of needle punctures until leaving a needle mark and tear. (Fig 3) The durability results of simulated skin showed statistically significant differences at the 0.1 level (Kaplan-Meier method). RTV-01 silicone rubber gave a higher durability rate and minor damage than RTV-02 and RTV-03. The RTV-01 silicone rubber punctured the needle 75 times until leaving a needle mark, while the vascular access sheath was inserted 8 times until leaving a tear. (Chart 1)

The durability results of simulated vascular showed RTV-03 a higher durability rate and minor damage than RTV-02 and RTV-01, with statistically significant differences at the 0.1 level (Kaplan-Meier method). RTV-03 was able to puncture the needle to the skin 70 times until leaving a needle mark, and the vascular access sheath was inserted 12 times until leaving a tear. (Chart 2)

Reliability test results from the three cardiologists using Intraclass Correlation Coefficient (ICC) model analysis

The ICC values from the three cardiologists by estimating the three types of silicone rubber that were used to create the simulation skin, ICC = 0.88 (95% CI = 0.70 – 0.96). For vascular simulation, ICC = 0.88 (95% CI = 0.70 – 0.96) the expert confidence assessment results indicated consistency between the three experts and that they were of excellent reliability.

Satisfaction survey results from the fellowship of Internal Medicine Cardiology using a 5-point Likert scale

Values of resistance during needle puncture and catheter insertion were realistic with mean average score of 4.22. Skin softness and elasticity were real with an average score of 4.33. The shape of the simulation model was maintained after the procedure with an average score

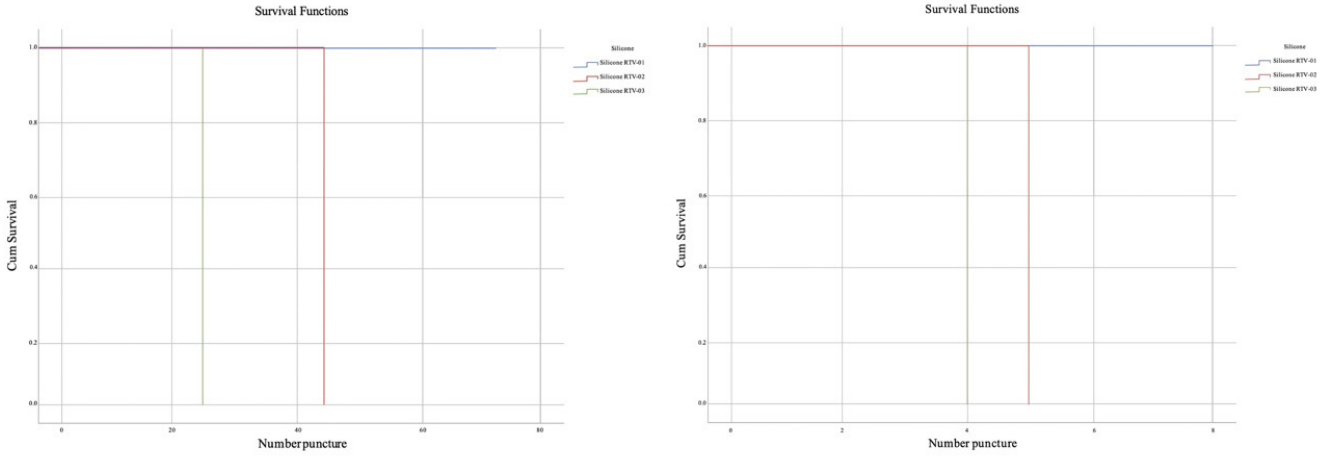

Chart 1. The simulated skin durability analysis (Kaplan-Meier) The number of needle punctures (left), and vascular access sheath insertion (right)

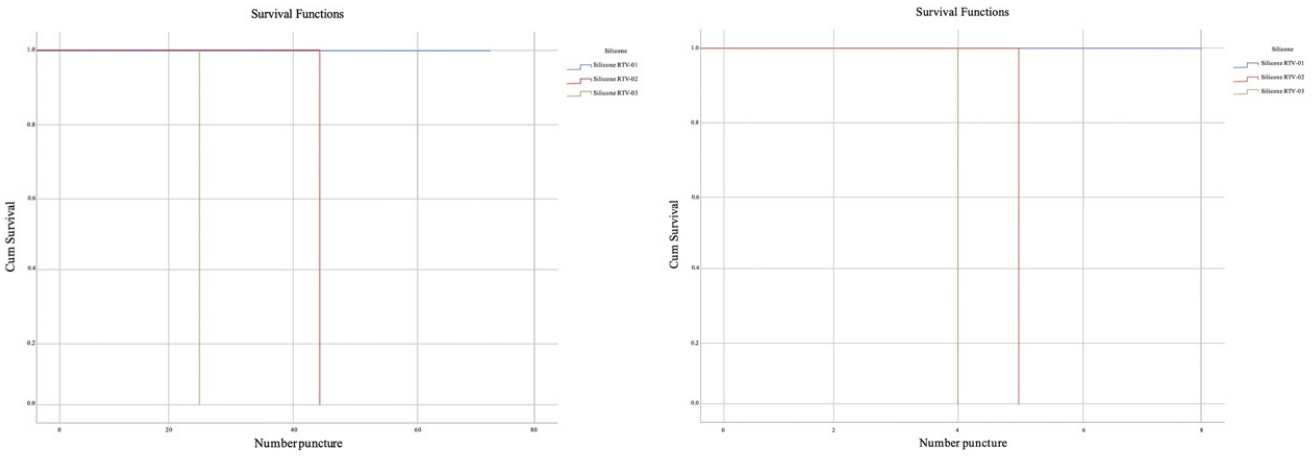

Chart 2. The simulated vascular durability analysis (Kaplan-Meier) The number of needle punctures (left), and vascular access sheath insertion (right)

of 4.50. The anatomical accuracy had an average score of 4.33. The blood flow was realistic with an average score of 4.33. The appearance of the model was suitable for usage with an average score of 4.78, and the total mean satisfaction of model performance had an average score of 4.41. These results showed that satisfaction with the performance of the model was at the highest level.

Satisfaction results showed that the simulation model enhanced the experience of the trainees with an average score of 4.67, reduced pressure before attending to an actual patient with an average score of 4.61 and increased confidence and readiness of the trainees with an average score of 4.56. Practicing the procedure in a controlled environment helped the trainees to prepare for potential problems with an average score of 4.50 and reduced the risk of performing the design on the patient with an average score of 4.61. The process for practicing the operation was realistic with an average score of 4.61, while total mean satisfaction with model usefulness recorded an average score of 4.59. These results showed that satisfaction with using the simulation model was at the highest level.

CONCLUSION

Simulation-based operational training is a valuable tool to increase learning efficiency and allows the fellowship trainees to practice operational techniques with the surgical equipment. Despite some simulators in cardiac catheterization training lacked realism due to their unrealistic form.[19,20] Lack of realism affects the surgical practice, which causes trainees to lack understanding of the procedure and the use of equipment. Therefore, researchers created a half-body human-size model to simulate an actual operation in which a patient is lying down on a surgical bed. Skin and vascular were mimicked as accurately as possible in terms of physical characteristics and capable of repetitive training. The materials can be reused and not accessible to tearing or leaving traces in operation. With

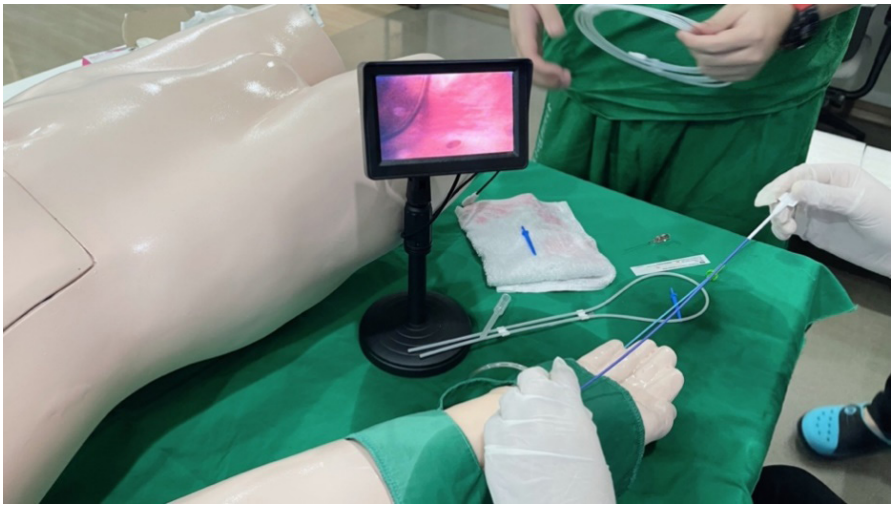

Fig 4. Transradial catheterization training

realistic and capability for repetitive training, transradial catheterization simulation motivates the fellowship trainees to become familiar with the equipment, gain a higher completion rate and increase confidence in treatment planning.

Recommendation

The study participants recommended developing a simulated pulse to the model that would make the practice of radial artery catheterization more realistic. A pulse would allow the participants to insert the needle correctly and reduce errors and damage to the skin and blood vessels.

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Histopathological and Clinical Features of Methotrexate-Associated Lymphoproliferative Disorders and Post-Transplant Lymphoproliferative Disorders

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ABSTRACT

Objective: To study histopathological and clinical features of methotrexate-associated lymphoproliferative disorders (MTX-LPD) and post-transplant lymphoproliferative disorders (PTLD).

Material and Methods: A retrospective study on 30 cases of MTX-LPD and 2 cases of PTLD from 2006 to 2021.

Results: By histopathology, the MTX-LPD group had 21 cases of lymphoma (MTX-Lymphoma) and 9 cases of reactive changes (MTX-Reactive). The PTLD group included diffuse large B-cell lymphoma and polymorphic PTLD (1 case each). The distinctive findings in MTX-Lymphoma and PTLD were association with Epstein-Barr virus (EBV) (8/12 cases, 66.7%) and CD30 positivity (13/18 cases, 72.2%). The MTX-LPD group had median MTX dosage of 10 mg/week, median MTX cumulative dosage of 2,613.75 mg, and median duration of MTX usage of 2,186 days. The 14 MTX-LPD and PTLD patients has median duration to response after the varied interventions of 47 days and the time to the first complete remission (CR) of 126 days. The MTX-Reactive patients had a significantly higher absolute lymphocyte count, younger median age, fewer B symptoms, higher rate of single site involvement, less extranodal involvement, shorter duration to response, less time to enter CR, and higher CR rate than the MTX-Lymphoma patients ($p < 0.05$).

Conclusion: Histopathology in MTX-LPD and PTLD patients can vary from reactive changes to lymphoma. EBV study and CD30 immunostaining help identify MTX-LPD and PTLD. History of MTX usage and other causes of immunodeficiency should be considered before diagnosing lymphoma. MTX discontinuation or reduction of immunosuppressant dosage are recommended before administrating combined chemotherapeutic agents in unresponsive cases.

Keywords: Methotrexate-associated lymphoproliferative disorders; post-transplant lymphoproliferative disorders; other iatrogenic immunodeficiency-associated lymphoproliferative disorders (Siriraj Med J 2022; 74: 575-589)

INTRODUCTION

Methotrexate (MTX) is an anti-metabolite of folic acid, used as an anti-neoplastic drug in many neoplasms (including malignant lymphoma) and as an immunosuppressive drug for autoimmune diseases.[1] But MTX has been associated with lymphoproliferative disorders (LPD) secondary to immunosuppression.[2-4] The revised 4th edition of the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues (WHO-HAEM4R) recognizes 4 types

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of immunodeficiency-associated LPD: post-transplant lymphoproliferative disorder (PTLD), lymphomas associated with HIV infection, lymphoproliferations associated with primary immune disorders, and other iatrogenic immunodeficiency-associated LPD that includes MTX-associated LPD (MTX-LPD).[5] Recently, the 5th edition of the WHO classification of lymphoid tumours (WHO-HAEM5) has been introduced as a review article since 22 June 2022 proposing a three-part nomenclature for LPD and lymphomas arising in the setting of immune deficiency/dysregulation: 1) histological diagnosis (hyperplasia, polymorphic LPD, mucocutaneous ulcer, or lymphoma classified as for immunocompetent patients); 2) viral association (EBV or KSHV/HHV8); and 3) immune deficiency/dysregulation setting (inborn error or immunity, HIV infection, posttransplant, autoimmune disease, iatrogenic/therapy-related, or immune senescence).[6]

The risk and type of MTX-LPD are varied upon the type of immunosuppressive agents used in addition to MTX, the degree of immune deficiency, and the nature of the underlying disorder being treated. Even though the disease is being more recognized now, there are no definitive diagnostic criteria and histologic categories. The diagnosis is based solely on LPD found in patients with a history of MTX usage who recover spontaneously after MTX discontinuation.[2,3] On the contrary, MTX-LPD patients with aggressive clinical courses at times fail to regress after MTX discontinuation.[7] It is unclear when MTX-LPD patients should receive chemotherapy after MTX discontinuation and the response may differ depending on the histopathology of MTX-LPD. Recently, The EBV real-time PCR test value in the peripheral blood[8] and programmed cell death-ligand 1 (PD-L1) expression in the lymphoma cells[9] were associated with spontaneous regression in MTX-LPD. Moreover, MTX-LPD patients previously treated with tumor necrosis factor-alpha or Janus kinase (JAK) inhibitors prior to MTX-LPD onset had more aggressive disease.[10]

This study aimed to investigate the histopathological and clinical features of MTX-LPD and PTLD, specifically of any distinct features to help pathologists identify these cases without knowing any clinical data, to clarify the differences in characteristics between MTX-LPD subcategories, and to identify MTX-LPD requiring chemotherapy upfront.

MATERIALS AND METHODS

Patients and tissue samples

This study was approved by the Institutional Review Board, Faculty of Medicine Siriraj Hospital, Mahidol University (SIRB) (Si 408/2021). For the MTX-LPD group, we compared the list of 14,139 patients with MTX treatment at Siriraj Hospital during January 2006 and May 2021 from the Department of Pharmacy and the Siriraj Informatics and Data Innovation Center to the archive list in the pathology laboratory information system (LIS) to identify the patients with a history of MTX usage and the histopathology of LPD. For the PTLD group, we searched for the cases diagnosed in the pathology LIS during the same period. We found MTX-LPD patients with lymphoma diagnosis (MTX-Lymphoma) 32 cases, MTX-LPD patients with reactive changes (MTX-Reactive) 21 cases, and PTLD patients 7 cases. The cases that lacked hematoxylin and eosin (H&E) staining and/or immunostained slides were excluded. Finally, we identified MTX-Lymphoma patients 21 cases, MTX-Reactive patients 9 cases, and PTLD patients 2 cases.

Patients were defined as having primary autoimmune disease by the information in the medical records. We defined MTX-LPD in patients receiving MTX at the time of diagnosis of LPD. In patients with underlying rheumatoid arthritis (RA), we evaluated the disease activity score in 28 joints count with 3 variables (DAS28-3) based on clinical and laboratory data 6 months prior to LPD diagnosis, which included the number of tender joints and number of swollen joints out of 28 joints, erythrocyte sedimentation rate (ESR), or C-reactive protein (CRP) calculated by http://www.das-score.nl.[11] All slides were re-evaluated by two pathologists (PN and SS) to record histopathologic features as listed in Table 1 and the disease diagnosis was made according to the WHO-HAEM4R.[5]

Immunohistochemical study (IHC) and Epstein–Barr virus-encoded small RNA (EBER) in situ hybridization (ISH)

The immunohistochemical study and EBV ISH were performed in an automated staining machine (The Ventana BenchMark XT automated slide-staining system, Tucson, AZ, USA). The IHC panels were listed in Table 2. Immunostained slides and the EBER ISH slides were re-evaluated by two pathologists (PN and SS).

Statistical analysis

Continuous demographic data were presented as the median and range. Categorical demographic data were presented as a percentage. Statistical analysis was performed to investigate the associations between the clinical and laboratory variables and the diagnosis of MTX-LPD by using Fisher's exact test or Mann-Whitney tests, as appropriate (SPSS Statistics, v. 18.0; SPSS, Inc.,

TABLE 1. List of histopathologic features recorded for evaluation in the study.

Histopathologic features	How to record
Residual lymphoid follicle	presence or absence
Tissue necrosis	presence or absence
Vascular invasion	presence or absence
Sclerosis	partial fibrous septa or absence
Histiocytic aggregation	presence or absence
Polymorphic reaction:	
Neutrophil	presence of ≥ 1 cell/HPF*
Eosinophil	presence of ≥ 1 cell/HPF
Plasma cells	presence of ≥ 2 cell/HPF
Hodgkin-Reed-Sternberg-like (HRS-like) cells	presence or absence

* HPF: high power microscopic field images were obtained under microscopy using a 40x objective lens with a field area of 0.159 mm^2.[5]

Chicago, IL, USA). A p-value < 0.05 was considered to be statistically significant. The cumulative 1-year and 5-year overall survival (OS) rates were analyzed using survival curves and were estimated by the Kaplan-Meier method and compared using log-rank tests.

RESULTS

Patient characteristics

In the 21 cases of the MTX-Lymphoma group, there were RA 17 cases, systemic lupus erythematosus 1 case, idiopathic exfoliative dermatitis 1 case, juvenile idiopathic arthritis 1 case, and idiopathic orbital inflammation 1 case. In the 9 cases of the MTX- Reactive group, there were RA 5 cases, antiphospholipid syndrome 1 case, ankylosing spondylitis 1 case, psoriasis 1 case, and Behçet disease 1 case.

Comparison of the clinical characteristics of the MTX-Lymphoma and MTX-Reactive patients (Table 3) showed that the MTX-Reactive patients had a significantly higher absolute lymphocyte count, younger median age, fewer B symptoms, a higher rate of single site involvement, less extranodal involvement, shorter duration to response, less time to CR, and a higher CR rate than the MTX-Lymphoma patients ($p < 0.05$).

Among the RA patients were DAS28-3 ESR available in 18 patients (13 MTX-Lymphoma and 5 MTX-Reactive) and DAS28-3 CRP available in 2 MTX-Lymphoma.[7] Interestingly, 5 MTX-Lymphoma patients had low RA disease activity (DAS28-3 CRP < 3.2) while 8 MTX-Lymphoma patients and 5 MTX-Reactive patients had moderate RA disease activity (DAS28-3 CRP > 3.2 but < 5.1) (Table 3).

The median MTX dosage at diagnosis of MTX-Lymphoma (7.5 mg/week) was lower than that of MTX-reactive (10 mg/week) ($p = 0.071$) but the median MTX cumulative dosage at LPD diagnosis of MTX-Lymphoma (2,880 mg) was higher than that of MTX-Reactive (665 mg) ($p = 0.167$). The median MTX usage duration at LPD diagnosis of MTX-Lymphoma (2,440 days) was longer that that of MTX-Reactive (410 days) ($p = 0.288$) (Table 3).

Of the 2 PTLD patients, one was a 59-year-old man when PTLD first developed. He had received a living-related kidney transplant for his idiopathic end-stage renal disease 8 years before PTLD diagnosis. He had received cyclosporin (125 mg/day), prednisolone (5 mg/day), and mycophenolic acid (720 mg/day) as immunosuppressants. His EBV PCR titer was less than 5,000 copies/μL and his anti-EBV IgG was more than 1:320. The other case was a 16-year-old man when PTLD first developed. He had received matched-unrelated donor hematopoietic stem cell transplantation for his aplastic anemia 90 days before PTLD diagnosis. He only received mycophenolate mofetil as an immunosuppressant at 40 mg/kg/day. His EBV PCR titer was less than 5,000 copies/μL and his anti-EBV IgG was 1:80.

TABLE 2. Immunohistochemical study panels used in the study.

Marker	Positive cell/other remark	Source	Antibody clone	Dilution
CD2	T-cell	BioGenex	AB75	1:200
CD3	T-cell	Novocastra	LN10	1:600
CD4	T-cell, helper	Ventana	SP35	RTU
CD5	T-cell	Cell Marque	4C7	1:100
CD7	T-cell	DAKO	CBC.37	1:100
CD8	T-cell, cytotoxic	Cell Marque	C8/144B	RTU
CD10	GCB	Novocastra	56C6	1:300
CD19	B-cell	Genova	LE-CD19	1:300
CD20	B-cell	DAKO	L26	1:2,000
CD21	Follicular dendritic cell	Cell Marque	2G9	1:100
CD23	Follicular dendritic cell	Cell Marque	MRQ-57	1:300
CD30	Activated lymphoid cell	Cell Marque	Ber-H2	1:100
CD56	NK cell	Cell Marque	123C3.D5	1:50
CD79a	B-cell	DAKO	JCB117	1:150
CD138	Plasma cell	DAKO	MI15	1:300
PD1	T-cell, follicular helper	Cell Marque	NAT105	1:50
CXCL13	T-cell, follicular helper	R&D	53610	1:300
βF1	T-cell receptor-beta	Thermo Scientific	8A3	1:20
γ-TCR	T-cell receptor-gamma	Thermo Scientific	γ3.20	1:20
TIA1	CGAP	Biocare	TIA-1	1:500
PAX5	B-cell	Cell Marque	SP34	1:200
c-myc	MYC transcription protein	Biocare	Y69	1:200
BCL2	Anti-apoptotic protein	DAKO	124	1:200
BCL6	GCB	Novocastra	LN22	1:200
MUM1	Plasma cell; non-GBC	DAKO	MUM1p	1:300
cyclin D1	MCL cell	Thermo Scientific	SP4	1:200
SOX11	MCL cell	Cell Marque	MRQ58	1:100
kappa	Plasma cell	Cell Marque	L1C1	1:1000
lambda	Plasma cell	Cell Marque	Lamb14	1:2000
TdT	Lymphoblast	Novocastra	SEN28	1:50
Ki-67	Proliferation index	DAKO	MIB1	1:300
LMP-1	EBV LMP	DAKO	CS1-4	1:150

Abbreviations: CGAP, Cytotoxic granule-associated protein; EBV LMP, EBV latent membrane protein; GCB, Germinal center B-cell; MCL, Mantle cell lymphoma; RTU, Ready to use

TABLE 3. Comparison of the clinical characteristics of MTX-Lymphoma and MTX-Reactive patients.

Parameter	MTX-Reactive (n = 9)	MTX-Lymphoma (n = 21)	P-value
Age in years, median (range)	47 (19–72)	62 (10–86)	0.02
Sex, n (%)			
Male	4 (44.4)	7 (33.3)	0.687
Female	5 (55.6)	14 (66.7)	
Presenting symptoms[a], n (%)			
Lymphadenopathy	7 (77.8)	12 (57.1)	0.419
Mass or nodule	0 (0)	6 (28.6)	0.141
Fever	0 (0)	3 (14.3)	0.534
Incidental findings	2 (22.2)	1 (4.8)	0.207
Others	0 (0)	4 (19)	0.287
B symptoms, n (%)	0 (0)	14 (66.7)	0.001
Focality			
Single	6 (66.7)	2 (9.5)	0.003
Multiple	3 (33.3)	19 (90.5)	
DAS28-3 ESR, median (range), n	3.64 (3.27–4.78), 5/18	3.59 (2.53–4.69), 13/18	0.424
DAS28-3 CRP, median (range), n	NA	3.245 (2.09–4.40), 2/2	NA
DAS28-3 ESR & CRP, median (range)	3.64 (3.27–4.78)	3.59 (2.09–4.69)	0.424
Absolute lymphocyte count in cells/µl, median (range)	1,938.30 (573.40–4,757.85)	780.80 (207.48–8,472.70)	0.028
Duration from primary autoimmune disease to LPD in days, median (range)	3,962 (79–4,787)	3,068 (385–7,421)	0.572
Extranodal involvement[b], n (%)	0 (0)	11 (52.4)	0.011
Bone marrow involvement, n (%)	(n = 2) 0 (0)	(n = 20) 4 (20)	1.000
MTX dosage at Dx in mg/week, median (range)	10.0 (7.5–20.0)	7.5 (2.5–17.5)	0.071
Duration of MTX usage in days, median (range)	410 (7–4,681)	2,440 (43–6,742)	0.288
MTX cumulative dosage at LPD Dx in mg, median (range)	665 (10–6,802.5)	2,880 (110–14,650)	0.167
Duration to response in days, median (range)	0 (0–329)	(n = 17) 65 (0–196)	0.024
Time to CR in days, median (range)	0 (0–485)	(n = 10) 177 (64-774)	0.013
Treatment response, n (%)		(n = 18)	
Complete remission	8 (100)	10 (55.6)	0.031
Not CR	0 (0)	8 (44.4)	
Relapse, n (%)	0 (0)	1 (4.8)	
1-year overall survival, n (%)	9 (100)	(n = 16) 10 (62.5)	0.057
5-year overall survival, n (%)	1 (100)	(n = 8) 2 (25.0)	0.333

DAS28-3, disease activity score 28-joint count–3 variables.
[a]some patients had more than one presenting symptoms.
[b]lung, liver, spleen, kidney, salivary gland, lacrimal gland, and brain.

Histopathology and immunohistochemistry

The histopathology findings and immunohistochemistry results are demonstrated in Table 4. The histopathology in the MTX-Lymphoma group (21 cases) included B-cell lymphoma 16 cases (76.2%), T-cell lymphoma 3 cases (14.3%), classic Hodgkin lymphoma (CHL) 1 case (4.8%) and EBV-associated LPD (EBV+ LPD) 1 case (4.8%). The most common histological pattern was DLBCL (8 cases, 38.1%). The other types included peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS) (3 cases, 14.3%), marginal zone lymphoma (MZL)/ mucosa-associated lymphoid tissue lymphoma (2 cases, 9.5%), and one each (4.8%) for CHL, lymphomatoid granulomatosis (LYG), intravascular large B-cell lymphoma (IVL), mantle cell lymphoma (MCL), B-lymphoblastic lymphoma (B-LBL) and EBV+ LPD. The last 2 cases could be diagnosed as unclassifiable B-cell lymphoma (case no. 15 & 16) because they lacked comprehensive immunostaining for a definite diagnosis.

Other histopathologic findings found in the lesions were as follows: residual follicles in 4 of 21 cases (19%), tissue necrosis in 8 (38.1%), vascular invasion in 4 (19%), sclerosis in 6 (28.6%), histiocytic aggregation in 3 (14.3%), HRS-like cell in 9 (42.9%), plasma cell reaction in 6 (28.6%), eosinophil reaction in 3 (14.3%), and neutrophil reaction in 1 (4.8%). The majority of lesional cells were large atypical lymphoid cells with varying numbers of admixed inflammatory cells among the lesional cells. The distinctive ancillary findings in MTX-Lymphoma and PTLD were EBV study and CD30 positivity. We found positive EBV LMP-1 (3 of 5 cases, 60%), positive EBER ISH (8 of 12 cases, 66.7%) and CD30+ cells (13 of 18 cases, 72.2%).

The histopathology in the MTX-Reactive group (9 cases) included mixed paracortical and follicular hyperplasia 5 cases (55.6%), follicular hyperplasia 2 cases (22.2%), paracortical hyperplasia 1 case (11.1%) and florid follicular hyperplasia 1 case (11.1%).

The histopathology in the 2 cases of PTLD included DLBCL 1 case and polymorphic PTLD 1 case.

Bone marrow status

Twenty MTX-LPD patients underwent bone marrow biopsy for staging. Two of them (10%) had diffuse involvement, another two (10%) minimal involvement by scattered lesional cells, and the remaining 16 cases (80%) negative marrow staging. One of the 2 PTLD patients had minimal involvement by scattered lesional cells and the other negative marrow staging.

Treatment and survival outcome

Details of the treatments and outcomes of the MTX-Lymphoma, MTX-Reactive, and PTLD cases are demonstrated in Table 5. The median follow-up was 394 days.

Only 6 of the 21 MTX-Lymphoma patients (28.6%) discontinued MTX as the first-line management (cases no. 1, 13, 15, 17, 20, and 21). Only 2 cases had spontaneous regression (cases no. 1 and 17) while 3 cases died (cases no. 13, 15, and 20; case no. 15 with progressive disease and case no. 20 with septic shock), and the other lost to follow-up (case no. 21). Among the cases that responded to MTX discontinuation alone, case no. 1 with histopathology of DLBCL had complete remission (CR) at 64 days after MTX discontinuation. Case no. 17 with histopathology of PTCL, NOS had partial remission at 20 days after MTX discontinuation; then computed tomography (CT) at 383 days after MTX discontinuation confirmed CR. Case no. 16 with unclassifiable B-cell lymphoma received prednisolone at the time of MTX discontinuation but lost to follow-up for 2 years but upon return to the hospital, physical examination showed no lymphadenopathy.

In this retrospective study, many patients did not have full records for the exact duration of the response after intervention, so the duration of the response shown in Table 3 refers to the evaluation after diagnosis. The median duration to response in 27 combined MTX-LPD and PTLD patients was 47 days (range, 0-329 days). The median duration to first CR in 20 combined MTX-LPD and PTLD patients was 126 days (range, 0-774 days).

Ten of the 21 MTX-Lymphoma patients (47.6%) discontinued MTX and simultaneously received combined chemotherapeutic agents (CMT) as the first-line management (cases no. 2-7, 9, 14, 18, and 19), 6 cases are still alive at the time of the report writing (5 without disease in cases no. 4, 5, 7, 9, and 14; while case no. 2 received only 1 cycle of R-CHOP and developed congestive heart failure but still alive with disease). Three cases died due to septic shock (cases no. 3, 6, and 18) and the other case lost to follow-up at 104 days after receiving the first-line management (case no. 19). There were 6 cases that achieved CR after MTX discontinuation and simultaneous CMT, including DLBCL 4 cases (cases no. 4, 5, 6, and 7), MZL 1 case (case no. 9), and B-LBL 1 case (case no. 14). The time to CR ranged from 110 to 344 days, with a median of 163 days. Two MTX-Lymphoma patients developed relapse of the disease (cases no. 6 and 9) based on examination of the bone marrow and lymph node, respectively. Case no. 6 had progressive disease after relapse and died. Case no. 9, however, could achieve CR after relapse and is still alive without disease.

Two of the 21 MTX-Lymphoma patients (9.5%) received only surgery as the first-line management without discontinuation of MTX or any CMT (cases no. 8 and 10).

TABLE 4. Histopathology and immunohistochemistry of MTX-Lymphoma, MTX-Reactive, and PTLD patients.

Case No.	Histopathology	Residual follicles	Tumor Necrosis	Vascular invasion	Sclerosis	Histiocytes aggregation	Polymorphic reaction	HRS-like cell	Tumor cell size	EBV-LMP-1	EBER	CD30
MTX-Lymphoma												
1	DLBCL	-	-	-	-	-	-	+	L	+	+	+*
2	DLBCL	-	-	+	-	-	PC	-	M to L	-	-	+*
3	DLBCL	-	-	-	P	-	-	-	M, L	-	-	-
4	DLBCL	+	-	-	P	+	Eo	+	M to L	NA	NA	+*
5	DLBCL	-	+	-	-	+	-	+	L	NA	-	+*
6	DLBCL	-	-	-	-	-	-	+	M, L	NA	NA	-
7	DLBCL	-	+	-	-	-	-	-	M, L	NA	NA	-
8	DLBCL	-	-	-	-	-	-	+	L	NA	NA	+*
9	MZL	+	-	-	P	-	PC, Eo	-	S	NA	NA	NA
10	MALT	+	-	-	P	-	PC	-	S, few L	NA	NA	NA
11	LYG	-	+	+	-	+	Neu	+	L	NA	+	+
12	IVL	-	-	-	-	-	-	-	L	NA	NA	NA
13	MCL	-	-	-	-	-	-	-	S to M	NA	NA	NA
14	B-LBL	-	-	-	P	-	-	-	M	NA	NA	NA
15	B-Lym	-	-	-	-	-	-	-	M to L	NA	NA	+*
16	B-Lym	-	+	-	-	+	-	+	M	NA	+	+*
17	PTCL, NOS	+	+	-	-	-	PC	-	S to M	NA	+	+*
18	PTCL, NOS	-	+	+	-	+	PC	+	S, M, L	NA	+	+*
19	PTCL, NOS	-	-	-	-	-	PC	-	S, M, L	NA	NA	-
20	CHL	-	+	-	P	+	Eo	+	S, L	+	+	+*
21	EBV+LPD	-	+	+	-	+	-	-	S to M, few L	NA	+	+*

TABLE 4. Histopathology and immunohistochemistry of MTX-Lymphoma, MTX-Reactive, and PTLD patients. (Continued)

Case No.	Histopathology	Residual follicles	Tumor Necrosis	Vascular invasion	Sclerosis	Histiocytes aggregation	Polymorphic reaction	HRS-like cell	Tumor cell size	EBV-LMP-1	EBER	CD30
PTLD												
22	DLBCL	-	-	-	-	-	-	-	M to L	NA	-	-
23	Poly-PTLD	-	+	-	-	-	-	-	S to M, few L	+	+	+*
MTX-Reactive												
24	F+PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
25	F+PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
26	F+PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
27	F+PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
28	F+PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
29	F	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
30	F	NA	NA	NA	P	-	-	-	NA	NA	NA	NA
31	PH	NA	NA	NA	-	-	-	-	NA	NA	NA	NA
32	F*	NA	NA	NA	-	-	-	-	NA	NA	NA	NA

*, focal positive; -, negative; +, positive

Abbreviations: B-LBL, B-lymphoblastic lymphoma/leukemia; B-Lym, B-cell lymphoma, unclassifiable; CHL, classic Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; EBER, Epstein–Barr virus-encoded small RNA in situ hybridization; EBV, Epstein–Barr virus; EBV LMP-1, Epstein-Barr virus latent membrane protein-1; Eo, eosinophil; F, follicular hyperplasia; F*, florid follicular hyperplasia; HRS, Hodgkin–Reed–Sternberg; IVL, intravascular large B-cell lymphoma; L, large; LYG, lymphomatoid granulomatosis; M, medium; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; NA, not available; Neu, neutrophil; P, partial fibrous septa; PH, paracortical hyperplasia; PC, plasma cell; Poly-PTLD, polymorphic post-transplant lymphoproliferative disorder; PTCL, NOS, peripheral T-cell lymphoma, not otherwise specified; S, small.

TABLE 5. Diagnosis, treatment, and outcome of MTX-Lymphoma, MTX-Reactive, and PTLD patients.

Case no. Age/Sex	Primary immune disease	Immuno-modulator	Biopsy site	Diagnosis	Focality	Extranodal involvement site	Clinical stage	IPI Score	1st-Line management	Duration to response (days)	Relapse	Outcome	Time to CR (days)	Mortality
1 68/F	RA	MTX, CQ	LN	DLBCL	Multiple	No	II	1	Off MTX	64	No	CR	64	Alive
2 58/F	RA	MTX, CQ	LN	DLBCL	Multiple	Kidney	III	1	Off MTX + R-CHOPx1	65	No	PR	NA	Alive
3 62/M	RA	MTX, Rituximab	Retroperi-toneal mass	DLBCL	Multiple	No	III	4	Off MTX + R-CHOPx6	90	No	PR	NA	Dead, Septic shock
4 70/M	RA	MTX, CQ, SSZ, LFM	LN	DLBCL	Multiple	No	II	2	Off MTX + R-CHOPx6	158	No	CR	158	Alive
5 71/F	RA	MTX, CQ	LN	DLBCL	Multiple	No	II	2	Off MTX + R-CHOPx6	40	No	CR	191	Alive
6 56/F	SLE	MTX, CQ	LN	DLBCL	Multiple	BM Liver	IV	3	Off MTX + R-CHOPx8	110	BM	CR	110	Dead, Septic shock
7 60/F	RA	MTX, CQ, AZA	Tonsil	DLBCL	Multiple	No	III	2	Off MTX + R-CHOPx8	163	No	CR	163	Alive
8 86/F	RA	MTX	Lung	DLBCL	Multiple	Lungs	IV	2	Lobectomy	0	No	CR	402	Alive
9 49/F	RA	MTX, LFM	Salivary gland	MZL	Multiple	Salivary gland	III	2	Off MTX + CVPx6+ CHOPx8	112	No	CR	344	Alive
10 69/M	IOL	MTX	Lacrimal gland	MALT	Single	Lacrimal gland	I	1	Lateral orbitotomy	0	No	Unknown	NA	Unknown
11 58/M	RA	MTX, SSZ, LFM	LN	LYG	Multiple	Lung NP	IV	2	PSL	120	No	PR	NA	Alive
12 75/F	RA	MTX	Skin	IVL	Multiple	Skin	IV	3	BSC	NA	No	Unknown	NA	Unknown

TABLE 5. Diagnosis, treatment, and outcome of MTX-Lymphoma, MTX-Reactive, and PTLD patients. (Continued)

Case no. Age/Sex	Primary immune disease	Immuno-modulator	Biopsy site	Diagnosis	Focality	Extranodal involvement site	Clinical stage	IPI Score	1st-Line management	Duration to response (days)	Relapse	Outcome	Time to CR (days)	Mortality
13 63/M	RA	MTX, CQ	BM	MCL	Multiple	Spleen	IV	3	Off MTX	NA	No	Unknown	NA	Death
14 10/F	JIA	MTX	Left orbital mass	B-LBL	Multiple	BM	IV	1	Off MTX + ThaiPOG-ALL	0	No	CR	142	Alive
15 54/M	RA	MTX, CQ	NP	B-Lym	Multiple	No	III	2	Off MTX	NA	No	PD	NA	Dead, progress
16 76/F	RA	MTX, CQ	LN	B-Lym	Multiple	No	III	4	Off MTX + PSL	NA	No	CR	774	Alive
17 62/M	RA	MTX, CQ	LN	PTCL, NOS	Multiple	No	III	3	Off MTX	20	No	CR	383	Alive
18 74/F	RA	MTX, CQ	LN	PTCL, NOS	Single	No	I	2	Off MTX + mini-CHOPx1	14	No	PR	NA	Dead, Septic shock
19 55/F	IED	MTX	LN	PTCL, NOS	Multiple	BM Liver	IV	3	Off MTX + CHOPx3	55	No	PR	NA	Unknown
20 69/F	RA	MTX, CQ	Liver	CHL	Multiple	Spleen Liver	IV	4	Off MTX	196	No	PR	NA	Dead, Septic shock
21 62/F	RA	MTX	Lung	EBV+LPD	Multiple	Lung Pleura	IV	1	Off MTX	78	No	PR	NA	Unknown
22 59/M	LRKT	CS, PSL, MA	Tonsil	DLBCL	Multiple	BM Spleen	IV	2	RI + CHOPx6	56	Tonsil	CR	71	Alive
23 16/M	MUD-HSCT	MM	LN	Poly-LPD	Single	No	I	NA	RI + Rituximabx4	NA	No	Unknown	NA	Dead, Septic shock
24 32/F	APS	MTX, CQ	LN	RL	Multiple	No	NA	NA	Off MTX	329	No	CR	329	Alive

TABLE 5. Diagnosis, treatment, and outcome of MTX-Lymphoma, MTX-Reactive, and PTLD patients. (Continued)

Case no. Age/Sex	Primary immune disease	Immuno-modulator	Biopsy site	Diagnosis	Focality	Extranodal involvement site	Clinical stage	IPI Score	1st-Line management	Duration to response (days)	Relapse	Outcome	Time to CR (days)	Mortality
25 19/M	Behçet's disease	MTX, PSL, HCQ	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Alive
26 47/F	RA	MTX, CQ	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Unknown
27 67/M	RA	MTX, CQ	LN	RL	Multiple	No	NA	NA	F/U	47	No	CR	47	Alive
28 72/F	RA	MTX, CQ, LFM	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Alive
29 47/M	RA	MTX, CQ, LFM	LN	RL	Multiple	No	NA	NA	Off MTX	14	No	CR	485	Alive
30 32/F	Psoriasis	MTX	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Unknown
31 56/F	RA	MTX, PSL, SSZ, LFM	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Alive
32 56/M	SpA	MTX, SSZ	LN	RL	Single	No	NA	NA	F/U	0	No	CR	0	Alive

Abbreivations: APS, anti-phospholipid syndrome; AZA, azathioprine; B-LBL, B-lymphoblastic lymphoma/leukemia; B-Lym, B-cell lymphoma, unclassifiable; BM, bone marrow; BSC, best supportive care; CHL, classic Hodgkin lymphoma; CQ, chloroquine; CR, complete remission; CS, cyclosporin; F/U, follow-up; DLBCL, diffuse large B-cell lymphoma; HCQ, hydroxychloroquine; IED, idiopathic exfoliative dermatitis; IOL, idiopathic orbital inflammation; IPI, international prognostic index; IVL, intravascular large B-cell lymphoma; JIA, juvenile idiopathic arthritis; LFM, Leflunomide; LN, lymph node; LRKT, living-related kidney transplant; LYG, lymphomatoid granulomatosis; MALT, mucosa-associated lymphoid tissue lymphoma; MCL, mantle cell lymphoma; MA, mycophenolic acid; MM, mycophenolate mofetil; MTX, methotrexate; MUD-HSCT, match-unrelated hematopoietic stem cell transplant; MZL, marginal zone lymphoma; NA, not available; Not CR = not in complete remission (PD, PR or SD); NP, nasopharynx; PD, progressive disease; Poly-PTLD, polymorphic posttransplant lymphoproliferative disorder; PR, partial remission; PSL, prednisolone; PTCL, NOS, peripheral T-cell lymphoma, not otherwise specified; RA, rheumatoid arthritis; RI, reduction of immunosuppressants; RL, reactive lymphadenitis; SD, stable disease; SLE, systemic lupus erythematosus; SpA, ankylosing spondylitis; SSZ, sulfasalazine; ThaiPOG-ALL, Thai pediatric oncology group treatment protocol for acute lymphoblastic leukemia.

Case no. 8 (aged 86 years) refused to receive any further treatment after surgery due to concerns about the side effects from CMT. The patient achieved CR 402 days after surgery as evaluated by CT and is still alive without detectable disease at 1,638 days after surgery. Case no. 10 lost to follow-up at 151 days after surgery before receiving any CMT according to the plan of treatment.

Of the 2 PTLD patients, the patient with DLBCL (case no. 22) achieved CR after immunosuppressants discontinuation and the completion of 6 cycles of CHOP. One year later, the patient had relapsed disease at the tonsils and received DA-EPOCH regimen for 6 cycles with rituximab for 4 courses. The patient achieved CR and is still alive. The patient with polymorphic PTLD (case no. 23) received a decreased dose of immunosuppressants and 4 courses of rituximab as the first-line management. Unfortunately, the patient died of a superimposed infection.

Of the 9 MTX-Reactive patients, only 2 cases discontinued MTX as the first-line management (cases no. 24 and 29); the others continued receiving MTX, but 2 of these 7 cases lost to follow-up. The 2 cases with MTX discontinuation and the other 5 cases with continued MTX had spontaneous regressions of the lymph node. The MTX-Reactive group tended to have a better survival rate when compared with the MTX-Lymphoma group, but without statistical significance (p = 0.065), and the PTLD group, with statistical significance (p = 0.034). The MTX-Lymphoma group tended to have better 1-year and 5-year survival rates than the PTLD group, but without statistical significance (p = 0.685) (Fig 1).

DISCUSSION

MTX-LPD is still not very well understood in terms of its definition, pathogenesis, prognosis, and treatment, but there are studies available in the literature to help us better understand and handle the disease. MTX-LPD is highly heterogeneous with various clinical presentations, histopathology, and disease progression. Our retrospective study categorized MTX-LPD according to histopathology and found that DLBCL was the most common pattern

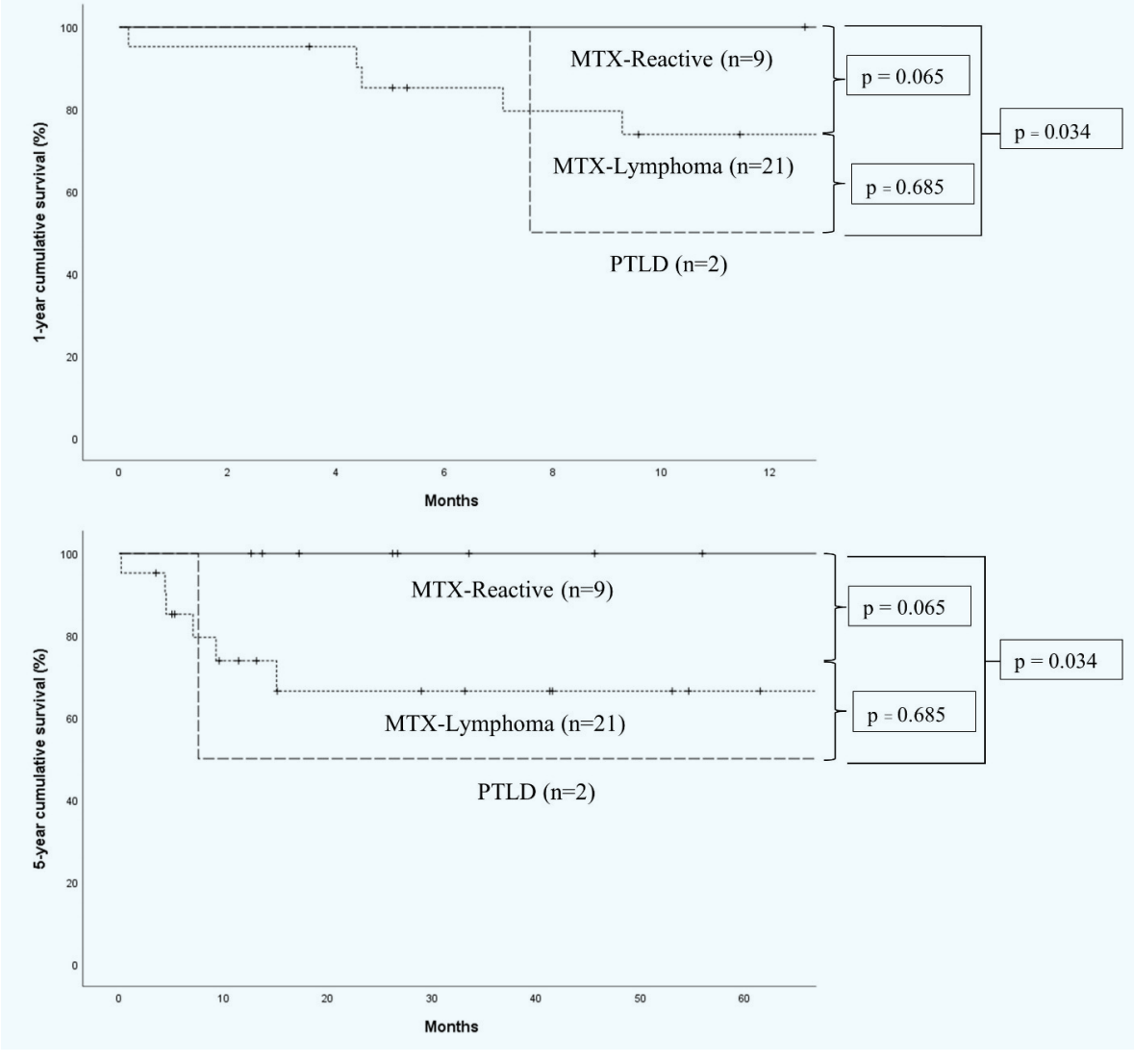

Fig 1. One-year and five-year cumulative survivals between patients of MTX-Reactive, MTX-Lymphoma, and PTLD.

in the MTX-Lymphoma group in concordance with previous reports,[2-4,12-16] but PTCL, NOS in our study was higher than in other reports; the reason is not known. Moreover, we encountered various kinds of the MTX-Lymphoma group, not only the common entities like DLBCL, PTCL, NOS, or CHL, but also MZL, MCL, and uncommon entities, like LYG and IVL.

Residual lymphoid follicles and vascular invasion were not common in our cases. However, tissue necrosis was frequently found in our MTX-Lymphoma cases; albeit, it can also be found in other lymphomas with aggressive behavior.[12] Histiocyte aggregation and plasma cell reaction were also frequently found in MTX-Lymphoma cases, but the significance and association with MTX have not been yet established.[12] Interestingly, Hodgkin-Reed-Sternberg-like cells were surprisingly common among the MTX-Lymphoma cases, but the significance and association with MTX have not been established yet either.[12]

A panel of immunostaining may give some additional information in these cases. In addition to the positivity of EBER ISH and/or EBV LMP-1 immunostaining, CD30 positivity in scattered large cells was found in many cases in our MTX-Lymphoma group, including DLBCL and PTCL, NOS. The 2 cases in our study (case no. 1 DLBCL and case no. 17 PTCL, NOS) showed regression of the lymph nodes after MTX discontinuation as the first-line management, corresponding to the expected clinical course of MTX-LPD.[2-4,12-16] Both cases showed EBV+ tumor cells by EBER and/or EBV LMP-1 as well as scattered CD30+ large cells, in agreement with previous reports.[13] These EBV association and CD30 positivity may help identify MTX-LPD,[12,13,17-19] but the distinction from de novo lymphoma with EBV association and/or CD30 positivity has not been studied.

Among the 9 MTX-Lymphoma cases in our study who had MTX discontinuation and who received CMT as first-line management, 3 of them died of septic shock that might or might not have been a consequence of the chemotherapy. One may wonder what would have happened if these patients had only MTX discontinuation so that they could have avoided the risk of superimposed infection following chemotherapy. As MTX-LPD is not a well-known entity, this may be the reason why MTX-Lymphoma patients often received a diagnosis of lymphoma instead of MTX-LPD, so that most patients received chemotherapy. This is problematic because the recommended management of MTX-LPD is to discontinue MTX and to wait for a response,[20] but there is no consensus about how long to wait for the response or how to assess the response. The response after MTX discontinuation reported in the literature varied from spontaneous to more than 8 weeks in some studies.[7,20] Our study had only 2 cases who responded to MTX discontinuation alone; one with histopathology of DLBCL that showed a response at 63 days after discontinuation. The other with histopathology of PTCL, NOS that showed a response at 20 days after discontinuation. These observations were in agreement with previous studies.[7,20] So, the administration of CMT in these patients may expose them to unnecessary risks from the chemotherapeutic agents. The MTX-Reactive group may not have even needed MTX discontinuation, since 5 of these cases showed a spontaneous regression despite continuing MTX.

The PTLD cases in our series did not have EBV serology tests, so we cannot be certain whether there was an EBV donor/recipient serology mismatch or not. Our polymorphic PTLD case died of superimposed infection despite the dosage of immunosuppressants being reduced and the patient receiving rituximab simultaneously according to the standard of care for LPD after hematopoietic stem cell transplant;[21] the superimposed infection seemed not to be related to the LPD treatment. The other PTLD case with histopathology of DLBCL was not EBV-associated; the patient had immunosuppressants discontinued and received R-CHOP according to the standard of care for LPD after solid organ transplant,[21] leading to CR. Despite relapse, the patient achieved second CR by salvage R-DA-EPOCH.

More than half of this group of patients had RA and interestingly about one-third of them had low disease activity index (DAS28-3) at LPD diagnosis. Thus, disease activity may not be useful to distinguish any group of patients. However, this study had a small sample size and may not be able to give adequate results.

Among the findings in the MTX-Reactive patients significantly different from MTX-Lymphoma patients, most of them held true for any reactive versus lymphoma comparisons. However, one of the interesting findings in our study was the absolute lymphocyte count. We found that the MTX-Reactive patients had a significantly higher median absolute lymphocyte count than the MTX-Lymphoma patients (1,938.3 vs. 780.8 cells/μl) ($p = 0.028$). This was in agreement with the report by Kurita et al. of peripheral blood lymphocyte counts of less than 1,000 cells/μl being found in their MTX-Lymphoma group more than in their MTX-Reactive group.[12] Saito et al. reported that the median lymphocyte count in persistent MTX-LPD was lower than in regressive MTX-LPD, with statistical significance,[22] but Nakano et al. reported that the median lymphocyte count in persistent MTX-LPD was higher than in regressive MTX-LPD,

though without statistical significance.[16] The absolute lymphocyte count may need further validation in a larger cohort to determine whether it can be used as one of the predictor for the regression of MTX-LPD.

Due to the small sample size of the study, we could not construct any link between the histopathology and the clinical features, the response after the discontinuation of MTX or immunosuppressants, or other clinical courses among the cases between the MTX-Lymphoma and PTLD groups. For the same reason, we could not find the pathognomonic histopathology for the pathologists to identify MTX-LPD without reviewing the medical records. We could not find a helpful clue to predict which case should respond to MTX discontinuation alone and which case would need upfront CMT unless we wait for a certain time to see the response of MTX discontinuation.[20] Further study should emphasize a comparison between MTX-Lymphoma patients with the same histopathology and, if applicable, a determination of clonality and genetic landscapes for any prominent features that would be helpful for pathologists to reach a diagnosis of MTX-LPD.

Based on this retrospective study, it is clear that we need in the future a comprehensive database to identify patients who have been receiving MTX so that this important piece of information can be linked to the laboratory information system for pathologists at the time of making a diagnosis. It could be very problematic if pathologists diagnose lymphoma without knowing that the patient is under MTX treatment, because the patient may be treated with chemotherapy for lymphoma without trying MTX discontinuation first. This could be harmful to the patient, because the side effects of chemotherapy may outweigh the benefit and can lead to death in some cases.

In conclusion, MTX-LPD and PTLD are highly heterogeneous diseases with a wide variety clinical spectrum and histopathology. Definitive diagnostic criteria and histopathologic categories for MTX-LPDs are yet to be established. Identifying MTX, other immunosuppressive drugs, and the immunodeficiency status of the patient are crucial in establishing the diagnosis. Immunostaining for CD30, EBV LMP-1, and EBER ISH study may be helpful to alert pathologists to consider MTX-LPD prior to making a diagnosis of lymphoma. MTX discontinuation is still the first-line management before the administration of chemotherapy in unresponsive cases of MTX-lymphoma, and similarly, lowering immunosuppressive drugs used in PTLD needs to consider with the hope of spontaneous regression of PTLD.

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Development and Validation of a New Design Self-assessment logMAR Visual Acuity Test ("Chudjane" iPhone- and iPad-based Application) in a Normal Eyes Population

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ABSTRACT

Objective: To validate and further design the "Chudjane" application (app), a new design self- assessment logMAR test for distance visual acuity (VA), by comparing the results against the use of a standard numeric ETDRS chart in normal eye population.

Materials and Methods: In total, 52 volunteers who had a normal eye exam and best-corrected VA score by numeric ETDRS (NE) chart equal to or better than 6/6 (logMAR score 0.00 or less) were included. The "Chudjane" app with 3 patterns of optotypes (Arabic numbers (AN), Tumbling-E (TE) and Landolt-C (LC)) was used twice to assess VA individually.

Results: The mean VA in each test NE, AN, TE, LC from the first round were -0.06, -0.10, -0.08 and -0.04, respectively compared to -0.07, -0.12, -0.09 and -0.05 from the second round respectively. Comparing results from the first and second round revealed that NE and LC had higher test-retest reliability (ICC=0.712, 0.789 respectively) than AN and TE (ICC=0.140, 0.495 respectively). For validity, result from NE was compared to each app test using the second round values. Modified Bland-Altman plot showed the mean differences (95% LOA) for NE-AN, NE-TE and NE-LC of 0.05 (-0.11 to 0.20), 0.02 (-0.11 to 0.15) and -0.03 (-0.19 to 0.13) respectively. Simple linear regression analysis of the difference (i.e. NE-AN, NE-TE and NE-LC) on NE showed that the difference did not depend on the NE value with slope close to zero.

Conclusion: The study demonstrated that by using the «Chudjane» application, LC had higher test-retest reliability and higher validity than TE and AN compared to the standard ETDRS chart.

Keywords: Self-assessment; visual acuity; mobile application (Siriraj Med J 2022; 74: 590-599)

INTRODUCTION

Visual acuity is the most commonly performed measurement of visual function in clinical practice. It is used to establish the need for a full evaluation of visual function and to inform the clinical decisions of ophthalmologists.[1]

Several optotypes are used in clinical practice, such as Arabic numbers (AN), Landolt-C (LC), and Tumbling-E (TE). Landolt-C and Tumbling-E are commonly used for assessing the vision of children, as well as for illiterate and non-English-speaking people; the results are determined by four orientations of the letters.[2-4] Landolt-C has been

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adopted as the primary standard optotype for visual acuity testing.[2] Arabic numbers (AN) are usually used in the clinical setting because patients are more familiar with this optotype, which comprise the numbers 0-9, which are typically recognized unequally.

Snellen's chart is the most common method for assessing visual acuity[1], mostly because it is easy and quick to use. However, it has some limitations, such as the nongeometric progression of the optotype size from line to line and the variable numbers of letters on each line.[5,6]

The ETDRS chart is the best-known logMAR chart, and is also used for most major research studies. It overcomes the limitations of Snellen's chart, as it incorporates geometric progression (step down of 0.10 log units from line to line) and equal numbers of letters on each line (5 letters per line). For the tests, each character is valued at 0.02 logMAR units per letter that is read correctly.[7]

The "Chudjane" test application (app) was designed such that it could be utilized by a user to self-assess their personal visual acuity and the app automatically summarized and reported the results of the visual acuity in logMAR units, together with the duration of the test.

We measured Visual Acuity at "distance" 4 meters (same as standard ETDRS) using both iPad & iPhone together, iPad as a chart at distance and iPhone as a remote control connected to iPad via Bluetooth that can select optotypes, characters and sizes as described in methods.

The limitations of standard ETDRS chart were memorization effect, patients could remember the fix characters on light box chart, and ETDRS character size changed in steps of 0.1 logMAR. This app was designed to prevent the memorization effect, and to be more fine-scale than the standard ETDRS chart.

In Thailand, like in most countries, smartphone use has been increasing every year (69.6% of the total population possessed a smartphone in 2018).[8] The development of healthcare applications has been increasing and there are now more than 100 visual acuity test applications available; albeit only a small number of them have been validated.[9]

Perera et al. performed a study in 2015 on the reliability and accuracy of 11 visual acuity applications on smartphones and reported that their accuracy varied from about 4.4%–39.9%.[10]

In the present study, the "Chudjane" test app was compared with a standard numeric ETDRS chart for assessing its validity agreement, reliability and duration of the test.

MATERIALS AND METHODS

Study participants

In total, 52 participants who were over 18 years old, healthy, Thai, could read Arabic numbers and who had no ocular disease by a slit lamp biomicroscope examination (except a refractive error that could be corrected by glasses to get best corrected visual acuity (BCVA) equal to or better than 6/6 (logMAR score 0.00 or less) which is the standard for normal eye visual acuity) were included in the study. The exclusion criteria included those who could not cooperate due to physical or mental disease and those who had a history of cycloplegic drugs or anesthetics allergy.

We validated the sample size based on an equivalence study (z-test), which indicated we required a sample size of 45 eyes to achieve 95% power with a significance level of 0.05 in order to detect an equivalence limit difference of 0.10 logMAR. We chose to use 0.10 logMAR because it represents a significant difference (95% confidence interval)[7] for test–retest variations. The standard deviation of difference was 0.20 logMAR, which was based on Bastrawrous et al.'s study.[1] Lastly, an allowance of 15% was made for drop-outs, so the calculated sample size was 52 eyes.

$$n = \frac{(z_\alpha + z_\beta)^2 \sigma^2}{(\delta - |\mu - \mu_0|)^2}$$

n = Sample size
α = Type-I error = 5% (Z = 1.645)
β = Type-II error = 5% (Z = 1.645)
σ = Standard deviation of differences
δ = Effect size ($|\mu-\mu_0|/\sigma$)
$\mu-\mu_0$ = Equivalence limit of differences

Ethics

The cross-sectional study adhered to the tenets of the declaration of Helsinki and was approved by the Ethics Committee of the Siriraj Institutional Review Board (COA no. Si 696/2018). Informed consent was obtained from all participants. The objectives of the study, examination process, benefits, and risks of the study were explained to all participants. All the participants gave their signed consent to participate.

"Chudjanes" test app

The app was written for the iOS platform, and this study used an iPad Pro 10.5" and iPhone 7 Plus for the testing. (Fig 1) The iPad was the main display device

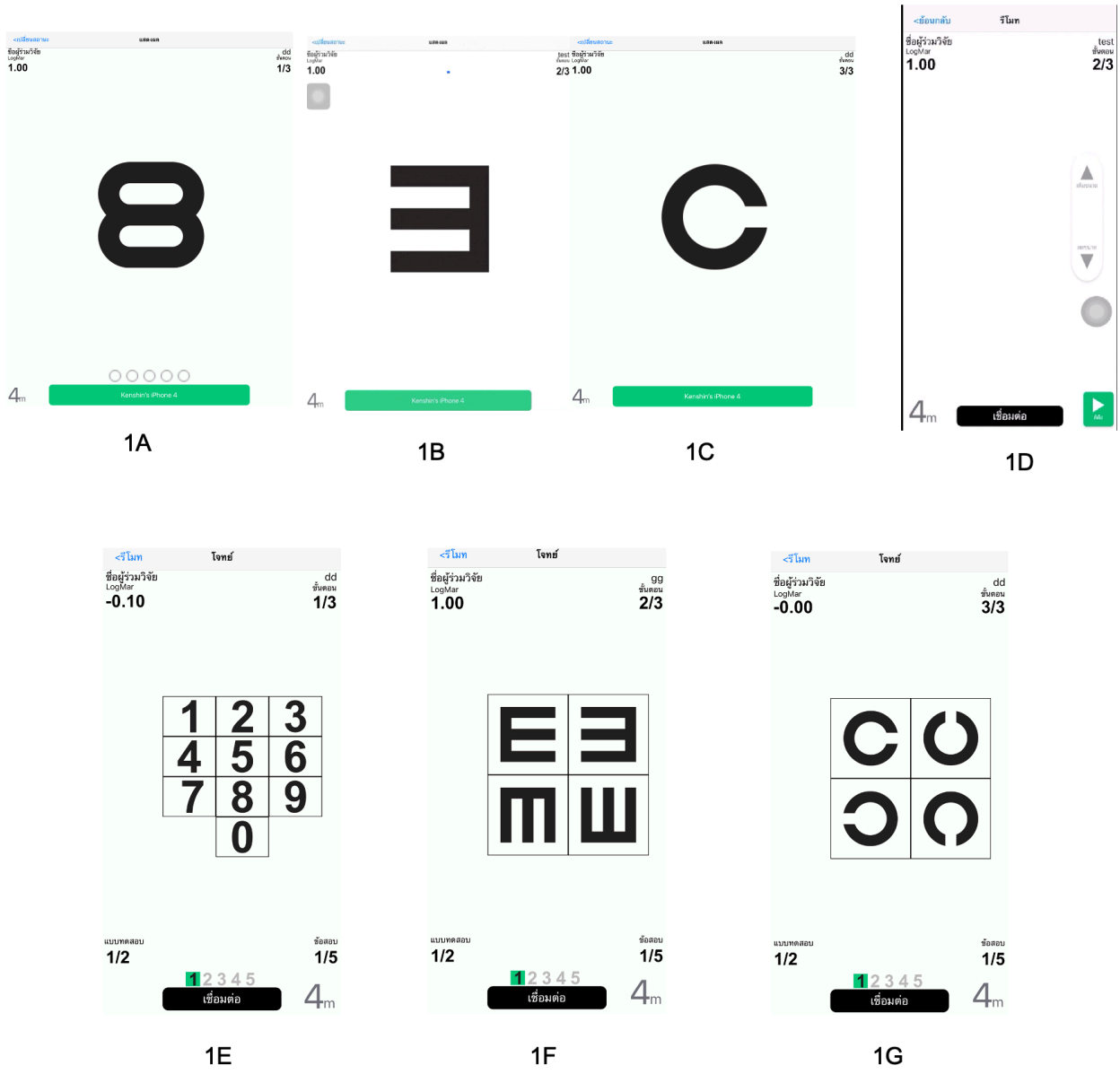


Fig 1. ***1A–1C***: iPad screen showing each optotype at 1.0 logMAR.
1D: iPhone screen in the remote controller mode while adjusting the size of the optotype.
1E–1G: iPhone screen in the remote controller mode while choosing the answers.

and displayed the optotypes located at 4 meters from the participant being tested. The iPhone served as a remote controller that was connected to the iPad via Bluetooth. (Fig 2) The brightness of the display on both devices was set to 100%.

The "Chudjane" test app was developed based on the logMAR chart, and was designed such that it could be utilized by a user to self-assess their personal visual acuity.

Three types of optotypes (Arabic numbers, Tumbling-E, and Landolt-C) were used and these could be selected as different options.

Arabic numbers (AN) consisted of the numbers 0 to 9, which were ordered in the same way as with a standard numeric ETDRS chart.

The Tumbling-E and Landolt-C optotypes consisted of 4 orientations of E or C (0º, 90º, 180º, and 270º).

This app was designed so that it could randomize all the characters of each optotype to prevent the memorization effect.

The size of the optotypes on the iPad could be controlled by the iPhone and the character size changed in steps of 0.1 logMAR for the Arabic numbers (AN) and 0.01 logMAR for the Tumbling-E (TE) and Landolt-C (LC) characters (allowing TE and LC to be more fine-scale than the standard ETDRS chart (NE) and AN by a factor of up to 10 times).

The app started with a single character displayed on the iPad, which equaled 1.0 logMAR on the ETDRS chart. The test subject used the iPhone to control and

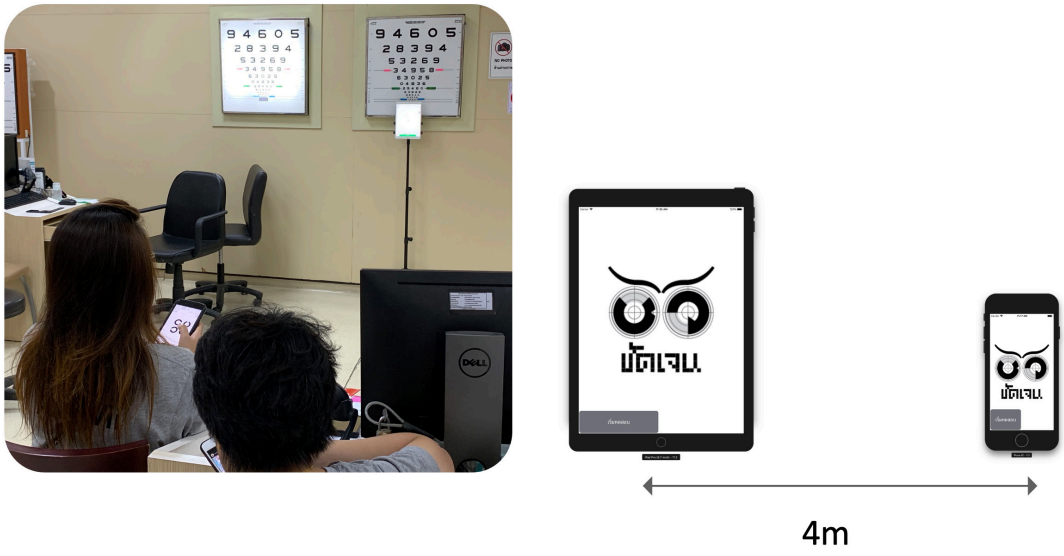


Fig 2. *Left:* The participant looks at the optotype on the iPad display, which was at a distance of 4 m away, while controlling the app using the iPhone7 plus. *Right:* Showing the distance between the iPad and iPhone, which was 4 m.

adjust the character size on the iPad display until it was the smallest size that they could still read, and then recorded the result on the app.

For the AN optotype, the app automatically selected the character size that matched the smallest character size in the test subject's records. Then it selected 5 numbers the same as on the Standard ETDRS chart to make 1 test set (equal to 1 line of the ETDRS).

For Tumbling-E (TE) and Landolt-C (LC) optotypes, the app used the smallest size that was recorded in the test subject's records and randomly selected 5 characters for each test set.

The test subject selected the answer on the iPhone that they believed matched the character on the iPad. After the first test set was completed, the results were automatically analyzed and the system response depended on the following 2 conditions.

In the first condition, if the test subject chose the correct answer for more than 2 out of the 5 characters (the subject got ≥3 correct) in each test set, the size of the 5 characters in the next test set was automatically reduced by 1 step. This condition was repeated until the correct answers in the last test set were less than three out of the 5 characters (the subject got ≤2 correct) or reached -0.3 logMAR. Then, the test was finished.

In the second condition, if the test subject chose the correct answer for less than 3 out of the 5 characters (the subject got ≤2 correct) in each test set, the size of the 5 characters in the next test set was automatically enlarged by 1 step. This condition was repeated until the correct answers in the test set were more than two out of the 5 characters (the subject got ≥3 correct). Then the character size in the last test set was reduced by 1 step, and the test was finished.

After the test subject had finished all the tests, the app automatically summarized and reported the results of the visual acuity in logMAR units, together with the duration of the test.

Scoring

The app scored the test subjects in 2 parts.

Part 1 Arabic numbers (AN): The score was 0.1 logMAR for each test set (5 characters), which equaled 1 line of the standard ETDRS chart. Each character was valued at 0.02 logMAR.

Part 2 Tumbling-E (TE) and Landolt-C (LC): Each character was valued at 0.002 logMAR because the progression of the optotypes was 0.01 logMAR for each test set (5 characters).

Testing protocol

1. All the participants underwent distant visual acuity testing using a back-illuminated 4-meters numeric ETDRS (NE) chart with the same chart and in the same environment. For all the tests, the presenting acuity was measured with the usual eyesight correction if worn. The duration of the test was also recorded.

2. The test subject's eyes were completely examined by a slit lamp biomicroscope to detect any abnormalities.

3. All the test subjects were given a demonstration on how to use the app.

4. The test subjects used the app to test their visual acuity by themselves.

5. After they had finished the first test (first round of testing) they were allowed to rest for about 15 minutes, and then they were tested again (second round of testing).

6. The test subjects' fundus was examined.

Statistical analysis

All the visual acuity measurements were converted to logMAR units, and the duration of the tests was converted to seconds. Data were summarized as mean ± SD. Test-retest reliability of NE, 3 App results (i.e., AN, TE and LC) from the first and second round was assessed via scatter plot, intraclass correlation coefficient (ICC, using a 2-way random effect model, absolute agreement and single measure) and classic Bland-Altman plot (X-axis = average from the first and second round as "true" value, Y-axis = difference) along with a simple linear regression line of the difference (Y) on the average (X).

Regarding the validity of app results compared to NE (gold standard), only data from the second round were used and analyzed by scatter plot and modified Bland-Altman plot (X-axis = NE, Y-axis = difference) with a simple linear regression line. Simple linear regression line and its 95% confidence band is applied to determine if the difference between NE and each app result depends on the true value. Slope (b) of the linear regression line that is close to zero indicates that the difference between NE and each app result does not depend on the true value. P-value of slope that is greater than 0.05 reveals that the population slope is zero.

Paired t-test was performed to test the difference in duration of test (second) from the first and second round.

Statistical analyses were performed using PASW 18 and MedCalc Statistical software version 19.6.4.

RESULTS

There were 52 subjects (44 females) aged 21 to 54 with mean of 33.3 ± 7.9 years.

The mean logMar in each test, i.e., NE, AN, TE, and LC, were -0.06, -0.10, -0.08, and -0.04, respectively, in the first round of testing, and were -0.07, -0.12, -0.09, and -0.05, respectively, in the second round of testing (Table 1).

For test-retest reliability of NE and app results from the first and second round, ICC for NE and LC were quite high (0.712 and 0.789) compared to only 0.140 and 0.495 for AN and TE respectively (Table 2). The classic Bland-Altman plot of the difference between the first and second round revealed that the all mean differences were very close to zero with the mean (bias) of 0.01, 0.02, 0.02 and 0.01 for NE, AN, TE and LC respectively (Fig 3). However, NE has the narrowest 95% LOA of -0.04 to 0.07 whereas AN had the widest 95% LOA of -0.19 to 0.23. TE and LC had similar 95% LOA of -0.13 to 0.16 and -0.11 to 0.12 respectively.

TABLE 1. Results of the tests from the first and second round.

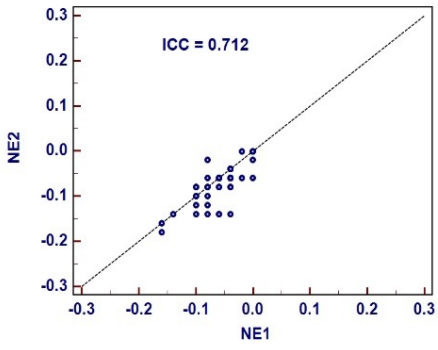
	logMAR: Mean ± SD	
	First round	Second round
Numeric EDTRS chart (NE)	-0.059 ± 0.036	-0.072 ± 0.041
"Chudjane test" app		
Numbers on app (AN)	-0.097 ± 0.076	-0.117 ± 0.085
Tumbling-E on app (TE)	-0.077 ± 0.073	-0.094 ± 0.074
Landolt-C on app (LC)	-0.037 ± 0.088	-0.046 ± 0.091

TABLE 2. Test-Retest reliability between results from the first and second round.

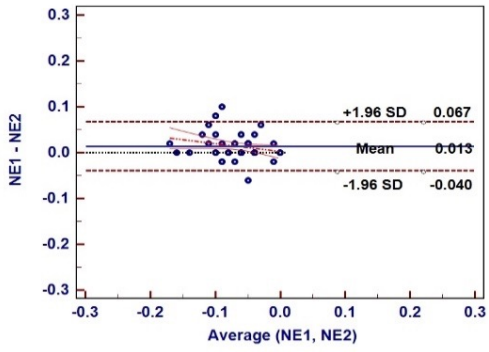
	ICC@	95% CI
Numeric EDTRS chart (NE)	0.712	0.492 to 0.836
"Chudjane test" app		
Numbers on app (AN)	0.140	-0.124 to 0.388
Tumbling-E on app (TE)	0.495	0.269 to 0.671
Landolt-C on app (LC)	0.789	0.662 to 0.871

@ ICC (Intraclass Correlation Coefficient): 2-way random effect model, Absolute agreement, Single measure

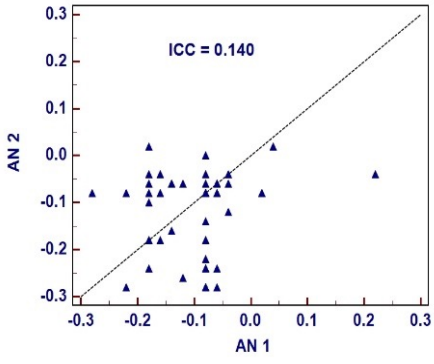
(a) Scatter plot: NE1, NE2



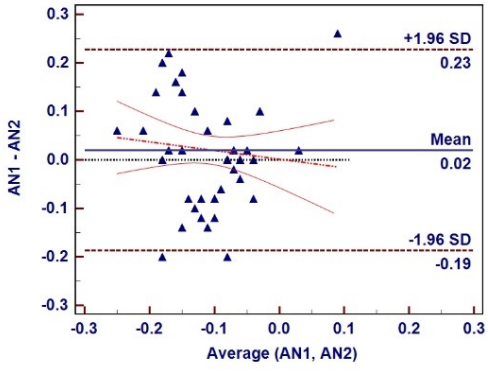
(b) Classic Bland-Altman plot: NE1, NE2



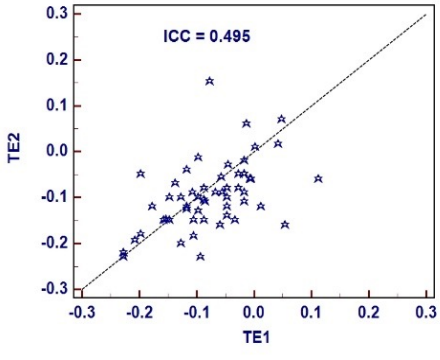
(c) Scatter plot: AN1, AN2



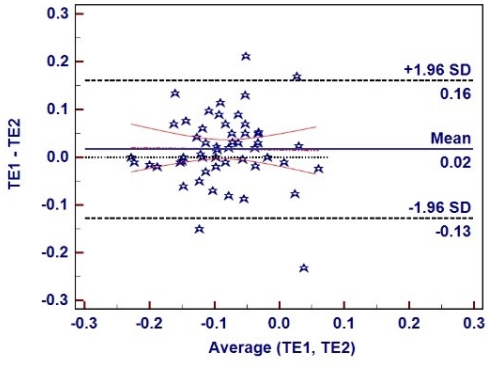
(d) Classic Bland-Altman plot: AN1, AN2



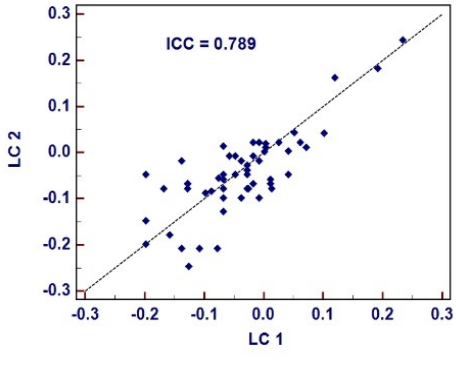
(e) Scatter plot: TE1, TE2



(f) Classic Bland-Altman plot: TE1, TE2



(g) Scatter plot: LC1, LC2



(h) Classic Bland-Altman plot: LC1, LC2

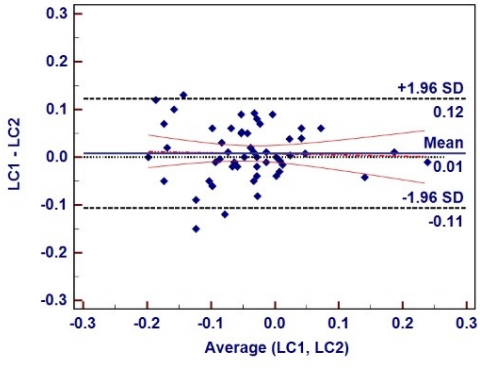


Fig 3. Test-retest reliability for NE, AN, TE and LC.

The logMAR of each app test was compared to NE. The mean difference (bias) of NE - AN, NE - TE, and NE – LC were 0.04, 0.02 and -0.02 respectively, in the first round of testing, and 0.05, 0.02 and -0.03 in the second round of testing (Fig 4). The upper limit of all 95%CIs of the mean difference was not greater than 0.1 logMAR. Validity of app test were assessed using values from the second round via scatter plots, modified Bland-Altman plots and simple linear regression line (Fig 5). The 95% LOA for AN were a little wider than those for TE and LC (-0.11 to 0.20, -0.11 to 0.15 and -0.19 to 0.13 for AN, TE and LC respectively). The simple linear regression line of the difference between NE and each app result (Y) on NE (X) revealed that all slopes were close to zero and not statistically significant (slope = 0.222, 0.214 and 0.028; p-value = 0.402, 0.342 and 0.917 for AN, TE and LC respectively, (Table 3) indicating that the difference between NE and each app result did not depend on the NE.

The average duration (seconds) of the NE, AN, TE, and LC tests were 33.8, 56.8, 162.2 and 145.7, respectively, in the first round of tests, and 32.0, 49.4, 155.7, and 143.9, respectively, in the second round of tests (Table 4). The duration of the test from the first round was a little higher than the second round with the mean difference of 1.8, 7.4, 6.5 and 1.7 seconds respectively and not statistically significant (p-values > 0.1).

There were no adverse events reported from performing any of the tests.

DISCUSSION

The number of individuals who use smartphones and tablets is increasing annually. There has also been a significant increase in smartphone and tablets usage among health professionals.[11,12] Currently, there are at least 100 vision test apps, but few have been validated.[9] This study aimed to develop and validate a new visual acuity app for use on the iPhone and iPad that anyone could use to test their visual acuity by themselves.

This Chudjane app was a new design that aimed to decrease the gap in the optotype size, which is currently ten times that of the standard EDTRS chart while still being based on logMAR scale progression to make the optotype size change more continuous. This would be expected to make the app more precise than tests performed using the old conventional chart. This app was also designed to decrease limitations of standard ETDRS chart that all fix characters were shown on light box chart. By using

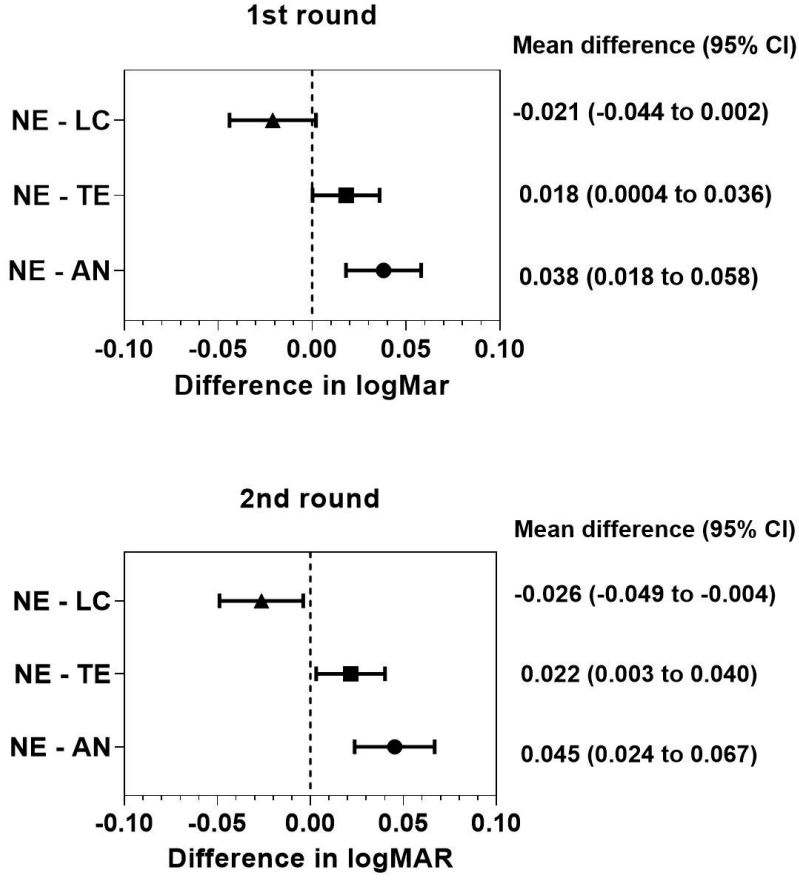


Fig 4. Comparison between NE and app results from the first and second round.

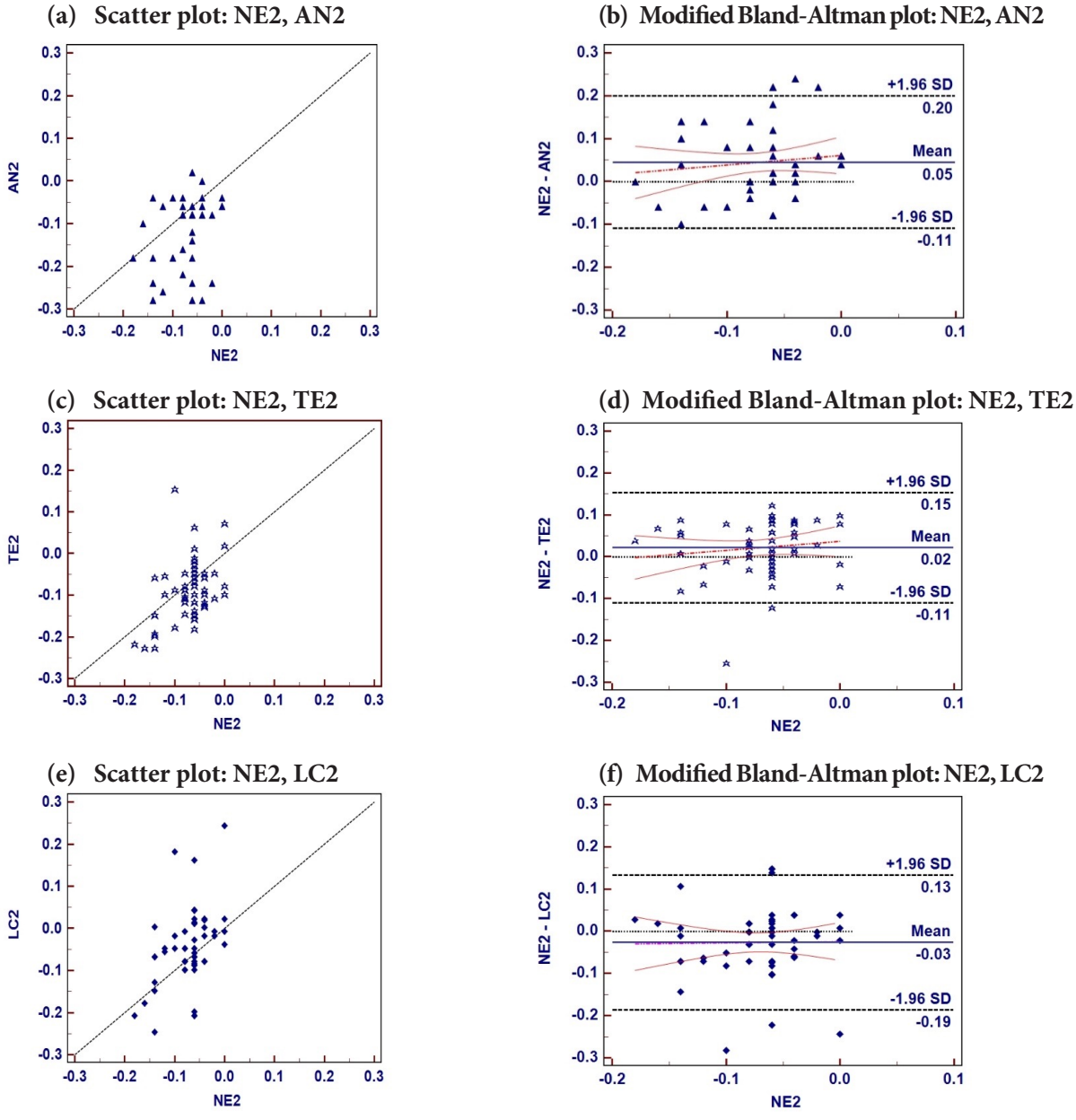


Fig 5. Validity of AN, TE and LC compared to NE (based on data from the second round).

TABLE 3. Difference between NE and app results from the first and second round.

		Difference		
		NE - AN	NE - TE	NE - LC
First round	Mean difference (Bias)	0.038	0.018	-0.021
	SD	0.074	0.065	0.084
Second round	Mean difference (Bias)	0.045	0.022	-0.026
	SD	0.079	0.067	0.081
	95% Limit of Agreement (LOA)			
	Mean – 1.96 SD (Lower)	-0.109	-0.110	-0.186
	Mean + 1.96 SD (Upper)	0.200	0.154	0.133
	Linear regression of difference (Y) on NE (X)			
	Slope	0.222	0.214	0.028
	(p-value)	(p=0.402)	(p=0.342)	(p=0.917)

TABLE 4. Duration of tests (second) in the first and second round.

	Mean ± SD		Difference (1st – 2nd round)	
	First round	Second round	Mean (95% CI)	P-value
Numeric EDTRS chart (NE)	33.8 ± 14.3	32.0 ± 13.3	1.8 (-1.5 to 5.3)	0.28
"Chudjane test" App				
Numbers on app (AN)	56.8 ± 36.9	49.4 ± 21.8	7.4 (-1.7 to 16.5)	0.11
Tumbling-E on app (TE)	162.2 ± 85.2	155.7 ± 87.4	6.5 (-21.8 to 34.8)	0.65
Landolt 'C' on app (LC)	145.7 ± 83.6	143.9 ± 121.7	1.7 (-33.5 to 36.9)	0.92

randomized characters, multiple optotypes and more fine-scale in this app should decrease memorization effect and limitations of standard ETDRS chart.

In this study, the results revealed that the mean VA in the normal eye population was less than logMAR 0.00 or better than 6/6, which is the standard for normal eye VA.

The mean VA with AN was the best. This could be due to possibly three causes: First, there may have been a memorization effect taking place since this optotype on the app was not random and also more closely resembled the standard EDTRS chart. Second, the AN size did not continuously decrease the same way as for TE and LC on the app, and there was a larger step difference between lines for AN. Finally, AN involves greater recognition acuity (i.e., reading a letter or Arabic number is easier), which could make it easier to guess the answer than TE and LC optotypes with greater resolution acuity. Several studies have reported that recognition acuity is higher than resolution acuity in low-vision subjects[13] and in normal-vision subjects.[14]

In terms of the mean differences and agreement when comparing each optotype in the app to NE, the results were all satisfactory (less than 0.1 logMAR), although agreement with TE and LC was less than for AN, which is of practical significance that the margin of error between the two charts is less than one complete line, which would result in similar patient care decisions based on the visual acuity measurements from either chart.[10,15] In terms of the mean difference of AN, it was the highest, which may result from a memorization effect occurring with AN, which was not the same as for the randomized optotypes, such as TE and LC. In a previous study[16], it was found that after a single VA test, a significant memory of a chart letter subset can be passively acquired and can persist for 10 days. The standard ETDRS back-illuminated light box had different brightness from the iPad digital screen display that use 100% brightness, also all the characters and lines were shown on the standard ETDRS light box but only a single character displayed on the iPad screen, these factors may cause memorization effect. The intraclass-Correlation-Coefficient (ICC) was highest in Landolt-C optotype because LC was optotype with greater resolution acuity, lesser memorization effect than NE, AN, TE[13-16] and LC and TE had smaller step difference between lines than AN in this app and NE.

The duration of the test using the app was longer than for the tests performed using the conventional ETDRS chart for all the optotypes, especially for TE and LC, since these were more detailed than for NE and AN by a factor of 10 times, and also, as many users were not acquainted with the use of the new app.

The strengths of this study include the fact that the test subjects tested themselves and that the LC and TE optotypes were random, which reduced subjective bias. There were three types of optotypes tested, so it is likely that the app can meet the needs of multiple populations and thus could be extensively applied.

The major limitation of this study to note is that the testing was only performed in a normal eye with best corrected visual acuity population. The use in clinical practice for an ophthalmic patient should also be studied in future research. The current design of the app interface on iPhone, which was used as a remote control, the AN optotypes that smaller than TE and LC may cause difficulty to use by people who have hyperopia and presbyopia. Still, this limitation could be eliminated by developing new voice to command features for the app. The app requires a little time for users to learn how to

use it. We will design it to be as user friendly as possible and further validation studies using other smart phones and screen type.

CONCLUSION

In conclusion, this study demonstrated that the "Chudjane" test application showed Landolt-C had highest test-retest reliability and validity compared to the standard ETDRS chart. This app is a new design self-assessment logMAR test for distance visual acuity suitable for normal eye population who have an iPhone and iPad as it allows the user to test and monitor their normal best corrected visual acuity by themselves. The use in clinical practice for an ophthalmic patient should also be studied in future research. Moreover, it has a potential to be a useful new tool to improve the services of physicians, such as screening and monitoring visual acuity.

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Conflicts of interest: None

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Visual Field Parameters and Pupil Size Measured through and Compared between Colored Contact Lenses and Clear Contact Lenses: A Prospective Comparative Pilot Study in Asian Population

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ABSTRACT

Objective: To compare visual field (VF) parameters and pupil size as measured through colored and clear contact lenses (CL) in Asian population.

Materials and Methods: Demographic data and CL prescription data were recorded during the baseline visit. Visual acuity and autorefraction were evaluated while patients wore clear CL, followed by slit-lamp examination and intraocular pressure measurements after CL removal. During the second visit, subjects were given dark brown-colored CL with their same prescription. Visual acuity and autorefraction were measured. Pupil size and VF tests were measured twice with the clear CL and the colored CL. Measurements through colored and clear CL were compared.

Results: Twenty-one volunteers (mean age: 31 years) were recruited. Refractive error varied from 0.00 to -7.00 diopters. Mean horizontal meridian measured through clear and colored CL was 134.8±7.8° and 133.3±4.7°, respectively. Mean vertical meridian measured through clear and colored CL was 100.1±3.8° and 100.4±2.5°. Mean total VF area measured through clear and colored contact lenses was 9,890.9±822.8° and 9,882.7±528.1°. There was no significant difference between clear and colored CL groups for horizontal meridian, vertical meridian, total VF area, or pupil size.

Conclusion: There was no significant difference in any evaluated parameters between clear and colored CL.

Keywords: Visual field parameters; pupil size; colored contact lenses; clear contact lenses; Asian population (Siriraj Med J 2022; 74: 600-603)

INTRODUCTION

Contact lenses (CL) are a commonly used method for correcting refractive error, and they are positioned directly on the cornea to improve light refraction. Since soft CL can cover the entire cornea, some people who do not require refractive error correction use colored CL to temporarily enhance or change their eye color. The use of colored CL for cosmetic purpose is becoming increasingly popular among Asian youths. According to the Study of the International Market for Contact Lenses conducted by Multi-Sponsor Surveys International LLC in 2010, the use of cosmetic tinted lenses among all CL wearers ranged from 24% in Taiwan to 39% in Singapore.[1] In the United Kingdom, cosmetic tinted CL are generally prescribed at the request of younger people (mean age: 27 years).[2] A 5 millimeter (mm) clear optical zone and a circumpupillary matrix of opaque colored dots are features of colored CL. These opaque colored dots may obscure peripheral vision or the visual field

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(VF), particularly when the pupil dilates in dim light and extends beyond the clear optical zone. Previous studies that evaluated this effect reported different results.[3-7] Accordingly, aim of this study was to measure visual field parameters and pupil size through both colored contact lenses and clear contact lenses and then to compare the results among Thai CL wearers. To clarify our concern about peripheral visual field that will obscure by these opaque colored dots especially in some situation such as driving at night that pupil size may get larger beyond clear optical zone.

MATERIALS AND METHODS

This prospective comparative study was conducted at the Department of Ophthalmology of the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand during February 2020 to May 2021. We enrolled healthy Thai CL wearers aged 18 – 39 years old with best-corrected visual acuity (BCVA) of 6/6 and spherical equivalent of less than 0.5 diopters. Patients with any ocular disease that could adversely affect peripheral vision or BCVA were excluded. Patients who had unavailable colored CL prescription were excluded. The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB) [Si 892/2019 (IRB3)], and informed consent was provides by all study participants before joining the study.

All study participants were evaluated over the course of two visits. During the first visit, demographic data and detailed CL prescription data were recorded. Visual acuity (VA) and autorefraction were evaluated while the CL were on the patient's eyes, followed by slit-lamp examination and intraocular pressure (IOP) measurements after the CL were removed. During the second visit, study subjects were given dark brown-colored contact lenses (Maxim® cocoa-colored contact lenses; Maxim Inter-Corporation LTD, Bangkok, Thailand) with the same prescription as that obtained from the patient at the first visit. VA and autorefraction were then reevaluated with the patient wearing the newly provided brown-colored CL.

In order to compare pupil diameter and VF parameters between clear and colored CL, patients were first randomly allocated to 1 of 4 testing sequence groups, as shown in Table 1. All measurements were made while CL were being worn. In all patients, pupil diameter was measured before performing VF measurements in each eye. In all patients and for all measurements, a colored CL was worn in one eye, and a clear CL was worn in the other eye. To control for carry-over effects, such as learning and sequencing, two rounds of VF measurement were performed with a 5-minute break between the two measurement rounds. The clear and colored CL were switched to the opposite eyes for the second round of measurements. All VF measurements were performed using an autokinetic Humphrey Field Analyzer (HFA; Carl Zeiss Meditec, Dublin, CA, USA). The HFA was set at standard illumination (III 4 e white) to measure the peripheral VF in 8 meridians (0, 45, 90, 135, 180, 225, and 270 degrees) in automatic pattern, and to give a kinetic 90-degree report. The means of all measured parameters were compared between the clear CL group and the colored CL group. The size of the pupil was measured by HFA before the visual field measurement was performed. The mean of the pupil size was compared between the clear CL group and the colored CL group.

Statistical analysis

SPSS Statistics (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical analyses. Paired *t*-test was used to compare all evaluated parameters between the clear and colored CL groups. Within group data are presented as mean plus/minus standard deviation. The result of the comparison between groups for each evaluated parameter is shown as mean difference between groups and 95% confidence interval. A *p*-value less than 0.05 was considered statistically significant for all tests.

RESULTS

Twenty-one volunteers (42 eyes) with an age range of 21 to 39 years (mean age: 31 years) were enrolled in this study. There were 20 females and 1 male. The refractive error among all patients varied from 0.00 to -7.00 diopters. There were 6 subjects in VF sequence group 1, and 5 subjects in each of the other 3 VF sequence groups. Only the VF and pupil measurement data collected during the second round of measurement were used for analyzed. VF and pupil measurement data are shown in Table 2 and Figs 1 - 4. The mean horizontal meridian measured through clear and colored CL was 134.8±7.8° and 133.3±4.7°, respectively. The mean vertical meridian measured through clear and colored CL was 100.1±3.8° and 100.4±2.5°, respectively. The mean total VF area measured through clear and colored contact lenses was 9,890.9±822.8° and 9,882.7±528.1°, respectively. The mean difference in the horizontal meridian, the vertical meridian, and the total VF area measured through clear and colored CL was 1.52 (p=0.372), -0.33 (p=0.648), and 8.25 (p=0.951), respectively. The mean pupil size measured through clear and colored CL was 4.53±0.80 mm and 4.72±0.75 mm, respectively. The mean difference in pupil size measured through clear and colored CL was -0.19 (p=0.136). There was no significant difference between the clear and colored CL groups for horizontal meridian, vertical meridian, total VF area, or pupil size.

TABLE 1. Sequences of visual field testing randomly assigned for each subject.

	Visual field 1		Visual field 2
Group 1	OD: Colored CL → OS: Clear CL	Rest 5 minutes	OD: Clear CL → OS: Colored CL
Group 2	OS: Clear CL → OD: Colored CL		OS: Colored CL → OD: Clear CL
Group 3	OD: Clear CL → OS: Colored CL		OD: Colored CL → OS: Clear CL
Group 4	OS: Colored CL → OD: Clear CL		OS: Clear CL → OD: Colored CL

TABLE 2. The horizontal meridian, vertical meridian, total area of visual field (VF) and pupil diameter measuring through clear and colored contact lenses (CL).

	VF Mean± SD		Clear CL – Colored CL	
	Clear CL	Colored CL	Mean difference (95% CI)	P -value
Horizontal meridian (degrees)	134.8 ± 7.8	133.3 ± 4.7	1.52 (-1.95, 5.00)	0.372
Vertical meridian (degrees)	100.1 ± 3.8	100.4 ± 2.5	-0.33 (-1.83, 1.17)	0.648
Total area of VF (degrees^2)	9890.9 ± 822.8	9882.7 ± 528.1	8.25 (-270.95, 287.45)	0.951
Pupil diameter (millimeters)	4.53 ± 0.80	4.72 ± 0.75	-0.19 (-0.46, 0.07)	0.136

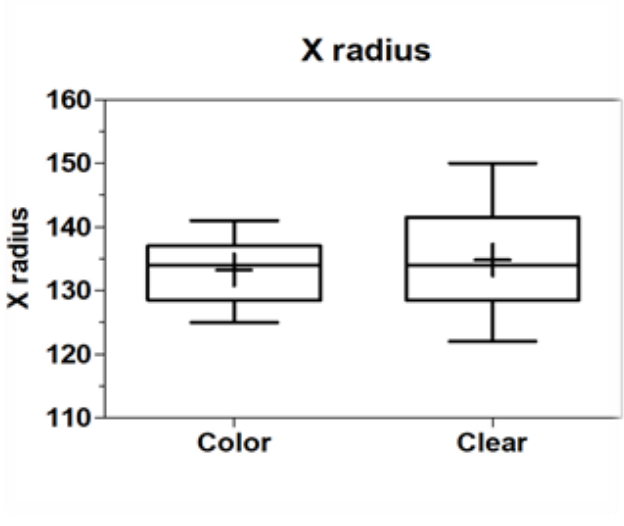

Fig 1. Comparing horizontal meridian of visual field between clear contact lenses and colored contact lenses (degrees).

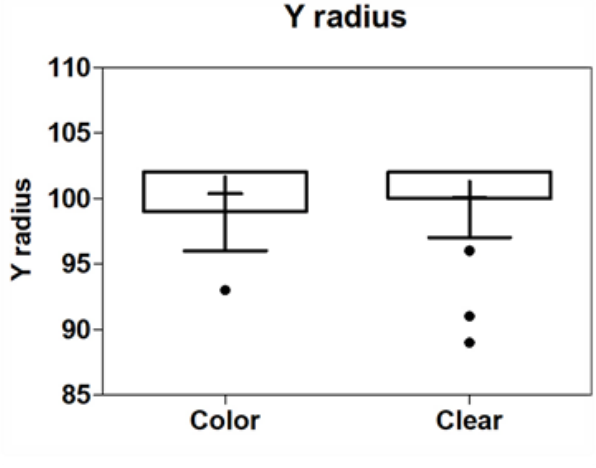

Fig 2. Comparing vertical meridian of visual field between clear contact lenses and colored contact lenses (degrees).

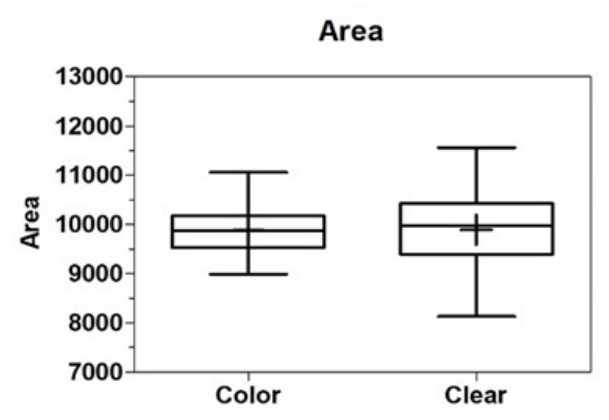

Fig 3. Comparing total area of visual field between clear contact lenses and colored contact lenses (degrees).

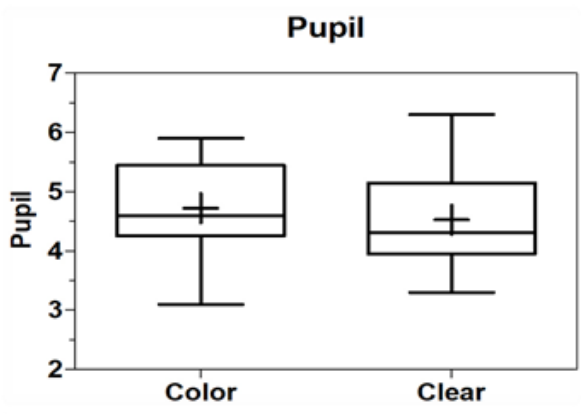

Fig 4. Comparing pupil diameter between clear contact lenses and colored contact lenses (mm).

DISCUSSION

Colored CL can temporarily change or enhance eye color using a matrix of opaque colored dots that surround a clear optical zone. Gauthier, *et al.* studied comfort, VA, and VF parameters in opaque tinted soft CL wearers, and they found no alteration of these parameters while wearing the evaluated the opaque tinted soft CL.[5] Trick, *et al.* found no significant effect of opaque tinted CL on VF using the 30-2 VF test.[6] In contrast, Insler, *et al.* reported that dot matrix CL could constrict both central and peripheral VF isopters by as much as 5 to 20 degrees as evaluated by Goldmann kinetic perimetry.[3] Josephson, *et al.* tested the 30-80 degree peripheral field of 10 patients and found at least 10 degrees constriction during colored CL wear.[4] In the present study, we used Maxim® cocoa-colored CL with a 5-mm clear optical zone and dark brown color at the circumpupillary area. We assumed these CL to have the darkest color available on the Thai market. We found the pupil diameter to be larger when wearing colored CL compared to that when wearing clear CL, the difference in pupil diameter between groups was not statistically significant. We also found no significant difference in any of the evaluated VF parameters between the colored and clear CL groups.

CONCLUSION

There was no significant difference in horizontal meridian, vertical meridian, total VF area, or pupil size as measured through and compared between clear and colored CL in Thai population. So the colored CL may not effect both on peripheral visual field and size of the pupil.

The main limitations of this study were its small sample size, the enrollment disparity between genders, and the fact that there is a difference in the design and diameter of the clear optical zone among different brands of colored CL.

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Conflict of interest declaration

All authors declare no professional or personal conflicts of interest, and no financial support from the companies that produce and/or distribute the devices, or materials described in this report.

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Malignancy of the Lymph Node: How General Practitioners and Pathologists can achieve a Definitive Diagnosis

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ABSTRACT

The lymph node plays an important role in the lymphatic spread of abnormal antigens from exogenous or endogenous sources, including infectious agents, foreign bodies, self-antigens, and malignant cells, by harboring various immune cells that react to abnormal antigens and their sources. This often leads to enlargement of the lymph node, also known as "lymphadenopathy." In this review article, malignancy of the lymph node is the main focus, especially regarding how general practitioners and pathologists can achieve a definitive diagnosis. The basic principle relies on the normal structure, cellular components, and functions of the lymph node as well as the types of malignancy found. Careful clinical history taking of any possible cause of lymphadenopathy warrants exclusion of any mimics of malignancy of the lymph node, including drug reactions and immunodeficiency states. An adequate cell or tissue sample allows pathologists to work efficiently by mastering the multimodality approach under good clinical collaboration. Effective communication between pathologists and physicians regarding relevant laboratory investigations should make it easier to diagnose a specific type of malignancy. This review article also focuses on how general pathologists handle cell or tissue samples by conventional morphologic evaluation and panels of immunohistochemistry so that general practitioners understand the diagnostic process and understand how to diagnose malignancy of the lymph node.

Keywords: Lymph node; malignancy; multimodality; morphology; immunohistochemistry; pathologic diagnosis
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INTRODUCTION

Malignancy of the lymph node can be divided into primary (the cellular components) and secondary or metastatic tumor, including leukemic infiltration. A primary malignancy of the lymph node is mostly the result of neoplastic lymphoid cell clone, best known as malignant lymphoma. Meanwhile, secondary malignancy is usually a metastatic tumor of nearby structures or at times an unknown primary site.[1] Leukemic infiltration of the lymph node is also a secondary malignancy, but terminology prefers to use infiltration (or involvement) to metastasis. However, this is uncommon prior to typical leukemic manifestation in general.[2] Leukemic infiltration, poorly-differentiated neuroendocrine carcinoma ("small cell carcinoma"), metastatic invasive lobular carcinoma, and other metastatic tumors may create difficulties in making a definitive diagnosis because they share morphologic similarities to malignant lymphomas. This particular group of tumor cells with small round cell morphology is often referred to as "small round-cell tumor (SRCT),[3,4] however, pathologists should pay close attention to the size of tumor cells because after provisional diagnosis,

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immunohistochemistry (immunostaining) can reveal diffuse large B-cell lymphoma (DLBCL). In such cases, discrepancy between provisional diagnosis of SRCT and final diagnosis of DLBCL can lead to a concern about the proficiency of the pathologist. In order to provide a definitive diagnosis of a malignancy in the lymph node, general practitioners and pathologists should understand the following topics.

Basic principles regarding the lymph node

The lymph node plays an important role in handling the lymphatic spread of abnormal antigens from exogenous or endogenous sources, including infectious agents, foreign bodies, self-antigens, and malignant cells, by harboring various types of immune cells that react to abnormal antigens. The process usually leads to enlargement of the lymph node, also known as "lymphadenopathy." Therefore, knowledge of the basic principles of normal structure, cellular components, and functions of the lymph node, as well as the types of malignancy commonly found in the lymph node should allow general practitioners and pathologists to make a definitive diagnosis.

I) Normal structure of the lymph node and types of malignancy found

Regarding primary or secondary malignancy of the lymph node, the normal structure of the lymph node can explain why tumor cells localized in lymph node sinuses tend to be metastatic tumors with lymphatic spreading. In addition, some lymphoma cells can have a lymph node sinus distribution mimicking metastatic tumor. When tumor cells are localized in the paracortex (interfollicular area or the area of the lymph node between the cortex and medulla), it can be suggestive of primary or secondary malignancy of the lymph node. Normally, the paracortex is predominantly occupied by T-cells (the "T-cell zone") but it is also the place where circulating cells enter the lymph node via postcapillary venules, commonly known as "high endothelial venules" (HEV) in immunology. Thus, not only T-cell lymphoma and B-cell lymphoma arising from a non-germinal center B-cell clone that are common in the paracortex, but also metastatic tumor cells with hematogenous spreading that can enter the lymph node via HEV in the paracortex. Certainly, leukemic cells can also reach the lymph node and cause lymphadenopathy via this route. The cortex, mostly occupied by B-cells and lymphoid follicles, is affected by malignant lymphomas, especially follicular lymphoma or other lymphomas arising from germinal center B-cells. Pathologists should be aware of the normal structure of the lymph node, especially the compartments commonly affected by various types of malignancy. The main challenge during microscopic examination of the lymph node is when tumor cells destroy normal structures of the lymph node (complete effacement of the lymph node) and the aforementioned clues cannot be used, leaving only morphologic evaluation of tumor cells as a possible solution.[1,5] (Table 1)

TABLE 1. Compartments of the lymph node and possible types of malignancy.

Compartment	Common malignancy	Less common malignancy
Lymph node sinus	Metastatic tumor with lymphatic spreading	Anaplastic large cell lymphoma, rare variant of diffuse large B-cell lymphoma, and Langerhans cell histiocytosis
Paracortex (interfollicular area)	T-cell lymphoma	B-cell lymphoma, metastatic tumor with hematogenous spreading, and leukemic infiltration
Cortex	Follicular lymphoma and others arising from germinal center B-cells	Nodular T-cell lymphoma (arising from T follicular helper cells) and follicular dendritic cell sarcoma
All compartments (diffuse effacement of the lymph node)	Any type of malignancy; definitive diagnosis depends on morphologic features and immunophenotypic findings of the tumor cells	

II) Cellular components of the lymph node and the types of malignancy commonly found

Cellular components of the lymph node are important for understanding why some types of malignancy occur more frequently. The lymph node cortex is predominantly a B-cell zone, while the paracortex is an T-cell zone. The medullary cords are predominantly occupied by plasma cells while the medullary and subcapsular lymph node sinuses are occupied by sinus histiocytes. Also, follicular dendritic cells mainly occupy the germinal centers of the lymphoid follicles. Less common in the lymph node are plasmacytoid dendritic cells and interdigitating reticulum cells. These cellular components of the lymph node work together to react to incoming antigens via lymphatics or HEV. The antigens arrive at the lymph node via lymphatics and trigger proliferation of sinus histiocytes so that the lymph node sinuses are dilated via accumulation of sinus histiocytes. At this point, without any detectable tumor cells, the enlarged lymph node caused by sinus histiocyte hyperplasia (or "sinus hyperplasia") is not diagnosed to have metastatic tumor.[1,5] Pathologists should avoid the terminology "sinus histiocytosis" because a general practitioner can get confused with "sinus histiocytosis with massive lymphadenopathy (SHML)" or "Rosai-Dorfman disease (RDD)." The author once found a patient who unfortunately received a course of local irradiation for an enlarged lymph node diagnosed as "sinus histiocytosis" because the radiotherapist misunderstood that it was "SHML" or "RDD." Fortunately, the patient was referred to Siriraj Hospital, the histologic slides were reviewed and a diagnosis of "sinus hyperplasia" of the lymph node was given instead of "sinus histiocytosis" to avoid misunderstanding as "SHML" or "RDD." Sometimes, lymphoma in a lymph node dissection specimen from patients who underwent tumor resection are overlooked as pathologists generally pay attention to metastatic tumors in lymph node sinuses.[6]

III) Functions of the lymph node and the types of malignancy found

The function of the lymph node in terms of immune reaction either helps or hampers diagnosis of malignancy. The most common immune reaction to tumor cells is involving tumor infiltrating lymphocytes (TIL), first described in malignant melanoma.[7] Therefore, assessment of TIL has been proposed in other types of malignancy as well.[8,9] In terms of morphologic evaluation of malignancy in the lymph node, TIL may lead to histologic features like lymphoepithelial carcinoma, indolent lymphoma, or T-cell rich variants in large cell lymphoma or classic Hodgkin lymphoma (CHL),[5] depending on the size, number, and morphology of tumor cells.

Epithelioid histiocytes (a pathology term) or activated macrophages (immunology term) sometimes intermingle with tumor and other immune cells in the lymph node. They can form tiny clusters, aggregates, sheets, or even granulomas, that lead to the wrong diagnosis of granulomatous lymphadenitis. In some places, where infectious diseases are common, coexisting tuberculosis and malignancy are found in the same lymph node.[10] When a lymph node with more clusters or aggregates of epithelioid histiocytes mixed with tumor cells, it can lead to the wrong diagnosis of granulomatous lymphadenitis. Therefore, pathologists should be aware of some tumors that have accompanying epithelioid histiocytes in clusters, aggregates, sheets, or even granuloma – germ cell tumor, CHL, and non-Hodgkin lymphoma (NHL) such as lymphoepithelioid lymphoma ("Lennert lymphoma"), a variant of peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma, T-cell/histiocyte rich large B-cell lymphoma, Burkitt lymphoma, and small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL).[5,11,12] Lastly, aggregates or sheets of epithelioid histiocytes can mimic metastatic carcinoma at times.

Tumor necrosis in the lymph node is occasionally seen with or without any preceding history of fine needle aspiration of the lymph node. At times, lymph node infarction can also occur. Careful evaluation for vascular occlusion can reveal tumor emboli or angiodestruction by tumor cells; the latter is more common in NHL.[5,13]

IV) Types of malignancy found in the lymph node

Malignant lymphomas and metastatic tumors are classified as primary or secondary malignancy, respectively. Malignant lymphomas at present are defined as a malignancy of lymphoid cells, which is different than in the past when it was a malignancy of the lymphoid tissue. Therefore, in the present classification of malignant lymphomas, we do not have histiocytic lymphoma, which was common in the 1960s according to the Rappaport classification for NHL. The different types of malignant lymphoma require clinical, morphologic, immunophenotypic, and genetic findings in order to make a definitive diagnosis according to the revised 4th edition of the WHO classification, published in 2017.[5] Malignant lymphoma can be divided into 3 types – B-cell lymphoma, T/NK cell lymphoma, and CHL – after excluding post-transplant lymphoproliferative disorder (LPD) and other iatrogenic immunodeficiency-associated LPD that can lead to varied pathologic findings, including any type of malignant lymphoma. A careful and complete clinical history review for immnosuppression is important in handling lymphadenopathy. Discontinuation of methotrexate or other immunosuppressive drugs can lead to improvement in clinical outcomes within one

week and spontaneous regression of the enlarged lymph node within three weeks.[5,14]

For general practitioners and pathologists, malignant lymphoma can be perplexing with 51 established entities plus six provisional entities of malignant lymphoma, according to the 2017 WHO classification.[5] A simplified version is presented in Table 2 with an emphasis on important issues that general practitioners and pathologists can help to manage efficiently for the lymphoma patients.

Regarding secondary malignancy of the lymph node, metastatic tumors are more common than leukemic infiltration or plasma cell myeloma (multiple myeloma).

TABLE 2. A simplified classification of malignant lymphomas for general practitioners and pathologists (modified from revised 4th edition of WHO classification, 2017)[5]

Clinical version	Morphologic version	Immunophenotypic version	Genetic version
Based on historical approach: Hodgkin vs Non-Hodgkin	Based on pattern: nodular vs diffuse	B-cell lymphoma - B-LBL - BL - DLBCL - FL - GZL - HGL - IVL - LPL - LYG - MCL - MZL - PBL - PEL - SLL	IGH gene, kappa Ig gene, lambda Ig gene BL: *MYC* DLBCL: *MYC; BCL2*; *BCL6; IRF4; MYD88*; FL: *BCL2* MCL: *CCND1* MZL: *BIRC3 (API2), MALT1, BCL10*
Based on clinical behavior: indolent, aggressive, or leukemic	Based on cell size: small vs large (including medium-sized).	T-cell lymphoma & NK/T-cell lymphoma - AITL - ALCL - ATLL - ENKT - HSTCL - ITL - MF/SS - PML - PTCL, NOS - SEBVT - SPTCL - T-LBL	TCR genes AITL: *RHOA, TET2, IDH2, DNMT3A, CTLA4* ALCL: *ALK, NPM1, BCL6, PTPN12, SERPINA1, CEBPB,* JAK/STAT pathway ATLL: *HBZ* HSTCL: *STAT5B*
Based on site of involvement: nodal vs extranodal	Based on nuclear features: blastic/blastoid vs mature	Hodgkin lymphoma - CHL - NLPHL	Clonal rearrangement of IGH gene by microdissection of LP & HRS cells
Based on identifiable causes: Breast implant-associated; chronic inflammation-associated; EBV-associated; *Helicobacter pylori*-associated; HHV-8-associated; HTLV-1-associated; MTX-associated; immunodeficiency-associated			

Abbreviations: AITL: Angioimmunoblastic T-cell lymphoma, ALCL: Anaplastic large cell lymphoma, ATLL: Adult T-cell leukemia/lymphoma, BL: Burkitt lymphoma, B-LBL: B-lymphoblastic lymphoma, CHL: Classic Hodgkin lymphoma, DLBCL: Diffuse large B-cell lymphoma, ENKT: Extranodal NK/T-cell lymphoma, nasal type, FL: Follicular lymphoma, GZL: Gray zone lymphoma (DLBCL vs CHL), HGL: High grade B-cell lymphoma (BL vs DLBCL), HRS: Hodgkin-Reed-Sternberg cells in CHL, HSTCL: Hepatosplenic T-cell lymphoma, IGH: Immunoglobulin heavy chain; ITL: Intestinal T-cell lymphoma, IVL: Intravascular large B-cell lymphoma, LP: LP cells in NLPHL, LPL: Lymphoplasmacytic lymphoma, LYG: Lymphomatoid granulomatosis, MCL: Mantle cell lymphoma, MF/SS: Mycosis fungoides/Sezary syndrome, MTX: Methotrexate; MZL: Marginal zone lymphoma, NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma, PBL: Plasmablastic lymphoma, PEL: Primary effusion lymphoma, PML: Primary mediastinal large B-cell lymphoma, PTCL, NOS: Peripheral T-cell lymphoma, not otherwise specified, SEBVT: Systemic EBV+ T-cell lymphoma of childhood, SLL: Small lymphocytic lymphoma, SPTCL: Subcutaneous panniculitis-like T-cell lymphoma, TCR: T-cell receptor, T-LBL: T-lymphoblastic lymphoma

Cell type and origin (primary site) of metastatic tumors are important because they help guide specific treatment and management of patients. Without any clinical information, pathologists attempt to determine cell type of the metastatic tumor by morphology, histochemistry, immunostaining, and a genetic approach. Usually, it is not difficult to diagnose metastatic tumors in the lymph node because tumor cells are found primarily in lymph node sinuses. The tumor cells tend to form aggregates or sheets as they form tight junctions with nearby tumor cells. Moreover, the morphology of most metastatic tumor cells is different from that of immune cells in the lymph node. However, at times, the metastatic tumor cells can look like lymphoid cells or other accessory cells in the lymph node such as histiocytes, follicular dendritic cells, and interdigitating reticulum cells. Leukemic infiltration, poorly-differentiated neuroendocrine carcinoma ("small cell carcinoma"), metastatic invasive lobular carcinoma, and a number of other metastatic tumors cause difficulties in making a definitive diagnosis because they share morphologic similarities to malignant lymphoma. An experienced pathologist should spend time looking for clues in histologic sections such as immature eosinophils (eosinophilic myelocyte) in leukemic infiltration, nuclear debris along the blood vessel wall in small cell carcinoma, and a large PAS+ cytoplasmic globule by histochemistry in invasive lobular carcinoma.

In case of metastatic carcinoma to the lymph node, common types include adenocarcinoma, squamous cell carcinoma, neuroendocrine carcinoma, urothelial carcinoma, clear cell carcinoma, mucoepidermoid carcinoma, anaplastic carcinoma, and metastatic carcinoma from special types of salivary gland tumors, thyroid gland tumors, pancreatic cancers, gynecologic cancers, etc. In terms of other non-hematologic malignancy, metastatic melanoma, germ cell tumor, and sarcoma are quite common. At present, several types of sarcoma can be diagnosed, even with a core needle biopsy.[15] Sarcomas tend to spread via the hematogenous route, however, sarcomas with potential for lymph node metastasis include rhabdomyosarcoma, epithelioid sarcoma, clear cell sarcoma, synovial sarcoma, and vascular sarcoma.[16] Another important issue is how to determine the nature of the obtained tissue for pathologic examination. Sometimes, it is difficult to distinguish between a lymph node and a soft tissue mass, especially when the subcapsular lymph node sinus cannot be identified. A core needle biopsy is certainly more challenging than an incisional biopsy, especially when the tumor extends into the perinodal soft tissue. In practice, a schwannoma may look like an enlarged lymph node clinically, but surgeons can identify it as a soft tissue mass during excision. Anyway, a number of schwannoma can be missed and submitted to pathology laboratory as "a lymph node." Pathologists usually demonstrate that it is schwannoma – not a lymph node. A core needle biopsy of a schwannoma in most published articles is claimed to lead to more accurate diagnosis than fine needle aspiration due to adequate tissue for evaluation.[17]

Before the advent of immunostaining, histochemistry was used quite frequently. It helps in identifying mucin production in metastatic adenocarcinoma, melanin pigments in metastatic melanoma, or PAS+ intranuclear inclusion in neoplastic plasma cells of lymphoplasmacytic lymphoma, other small B-cell lymphoid neoplasm with plasmacytic differentiation, or even nodal involvement by plasma cell myeloma (multiple myeloma). However, in daily pathology practice at present, a panel of immunostaining is preferred and some pathologists have no experience to use histochemistry for identifying certain materials as mentioned above. Anyway, histochemistry is still worthy in places with limited resources.

A panel of immunostaining can be used in different morphologic settings to determine cell types in a malignancy of the lymph node. The principle is to apply a commercially available antibody specific to an antigen of interest in relation to tumor cells with the hope that, after a panel of antibodies, the immunophenotypic findings can be gathered and interpreted with morphologic correlations to achieve a definitive diagnosis of the type of malignancy. Tables 3 to 7 demonstrate panels of immunostaining proposed for use in various morphologic settings. If the clinical impression and morphology support each other, a pathologist can order a marker specific to the suspected tumor. For example, immunostaining for CD56 (neural cell adhesion molecule or NCAM) can be used in a suspected case of neuroblastoma. However, the expected negative marker, such as vimentin, should be included in the immunostaining panel because CD56 is not specific for neuroblastoma. The author once had a case of intra-abdominal mass in a child who was clinically suspected to have neuroblastoma. The marrow sample showed small blue cells that could be neuroblasts. Only immunostaining for CD56 was performed and tumor cells were positive for CD56 but the serum neuron specific enolase (NSE) level was not elevated that was unusual for metastatic neuroblastoma. So, exploratory laparotomy was performed to remove the tumor mass that was proven to be a sarcoma, probably embryonal rhabdomyosarcoma, supported by positive markers for muscle differentiation, vimentin, and CD56. It has been well documented that CD56 can be positive in a

TABLE 3. Panels of immunostaining proposed for use in undifferentiated neoplasm with large cell morphology (at least 3 times small lymphocyte in size) in the lymph node.[5,31]

Marker	Positive in	Remarks
First screening panel of immunostaining		
AE1/AE3	Epithelial tumors & epithelioid variant of sarcomas	Dot positive in neuroendocrine carcinoma (need to view at 40x magnification)
CD45 (leukocyte common antigen, LCA, or common leukocyte antigen, CLA)	Lymphoma & some leukemia	Can be negative in some lymphoma cells but when positive, a few positive tumor cells should be kept for hematologic malignancy
S-100	Melanoma, LCH, histiocytic sarcoma	Need both nuclear and cytoplasmic staining for positivity
Second screening panel of immunostaining (after failed first panel)		
ALK	ALK+ large B-cell lymphoma	
CD30	ALCL & lymphocyte-depleted CHL	
CD56	Large cell neuroendocrine carcinoma	
CD68	Histiocytic tumors & monoblastic sarcoma (leukemic infiltration by monoblasts)	
CD138	Plasmablastic lymphoma	
EMA	Epithelioid variant of sarcoma	
MPO	Myeloid sarcoma (leukemic infiltration by myeloblasts)	

Abbreviations: ALCL: Anaplastic large cell lymphoma, CHL: classic Hodgkin lymphoma, LCH: Langerhans cell histiocytosis

number of normal cells and several kinds of tumors, including embryonal rhabdomyosarcoma.[18] Thus, a good immunostaining panel should include not only a positive marker but also a negative one for tumors listed in the differential diagnosis.

Tumor markers do not have ideal specificity so that a complete investigation for primary sites is still needed. For example, NKX3.1 is believed to be a marker of prostatic origin in metastatic tumors, but only one out of 349 non-prostatic tumor tissue tested positive for NKX3.1 and that was a case of invasive lobular carcinoma of the breast.[19] Even CD45 (leukocyte common antigen), which is regarded as a highly specific marker in the hematolymphoid neoplasm, there are only seven definitive cases from five reports to date of CD45 expression on non-hematologic malignancy, including one primitive sarcoma (most probably rhabdomyosarcoma), four neuroendocrine carcinomas (including small cell carcinoma), one undifferentiated large cell carcinoma, and one NUT carcinoma; three cases were lymph node metastasis.[20-24] Panels of immunostaining as shown in Tables 3 and 4 provide both positive and negative results that should not have any conflicting immunophenotype. For example, AE1/AE3+ carcinoma cells should not have CD45 expression. When AE1/AE3+ CD45+ tumor cells are detected, a search for any technical error should be performed before acceptance of such an abnormal phenotype (AE1/AE3+ carcinoma with aberrant CD45 expression or CD45+ lymphoma with aberrant AE1/AE3 expression). Technical errors can be the cause of abnormal expression when immunostaining is performed manually, such as wrong slide labeling, applying wrong antibody in immunostaining, contamination of antibody by other antibodies during preparation of primary antibody for use, and interpretation of positive tissue control as the result of the test. All these technical errors can be resolved by using the fully automated immunostainer, except the last one that is caused by the pathologist who looks at positive tissue control placed on the same slide of the tested tissue. To prove that the tumor cells have aberrant expression, the pathologist reviews the histologic section and decides the type of malignancy. For example, if the

TABLE 4. A panel of immunostaining proposed for use in undifferentiated neoplasm with small cell morphology (1-2 times small lymphocyte in size) in the lymph node.[4,31]

Marker	Positive in	Remarks
First screening panel of immunostaining		
AE1/AE3	Epithelial tumor & epithelioid variant of sarcomas	Dot positive in small cell (oat cell) carcinoma (need to look at 40x magnification)
CD45 (leukocyte common antigen)	Lymphoma & some leukemia	Can be negative in some lymphoma cells but when positive, even a few positive tumor cells, should keep work-up for hematologic malignancy
S-100	Melanoma (small cell variant)	Need both nuclear and cytoplasmic staining for positivity
Second screening panel of immunostaining (after failed first panel)		
CD33	Myeloblastic infiltration	
CD34	Leukemic infiltration	
CD56	Neuroblastoma, embryonal rhabdomyosarcoma, BPDCN	
CD99	EWS/PNET	
CD123	BPDCN	
CD138	Nodal plasmacytoma	Nodal involvement by PCM
MPO	Myeloblastic infiltration	
TdT	Lymphoblastic leukemia/lymphoma	

Abbreviations: BPDCN: Blastic plasmacytoid dendritic cell neoplasm, EWS/PNET: Ewing sarcoma/Primitive neuroectodermal tumor, PCM: plasma cell myeloma (multiple myeloma)

morphology is that of carcinoma, then the tumor cells have aberrant CD45 expression. But if the morphology is that of malignant lymphoma, then the lymphoma cells have aberrant AE1/AE3 expression. If the morphology is not conclusive of any type of malignancy, then more markers are needed to support a diagnosis of malignancy. For example, if the AE1/AE3+ CD45+ tumor cells express CD20, CD10, CD79a, PAX5, MYC, BCL2, and BCL6, but are negative for EMA, CD3, CD5, and MUM1, then the diagnosis should be DLBCL with germinal center B-cell as the supposed cell of origin with triple protein expression of MYC, BCL2, and BCL6 proteins, and aberrant AE1/AE3 expression. But if the AE1/AE3+ CD45+ tumor cells express CK8/18, TTF-1, CK7, CD56, chromogranin, and synaptophysin but are negative for CK20, p40, p63, CD3, CD20, CD30, CD138, and MUM1, then the diagnosis should be metastatic carcinoma, possibly primary pulmonary large cell neuroendocrine carcinoma with aberrant CD45 expression.

When there is a malignancy of the lymph node, tissue samples should be handled properly so that pathologists can make a definitive diagnosis. However, with limited resources, general pathologists try to separate reactive conditions from the neoplastic process to provide a possible diagnosis of the type of malignancy found in the lymph node such as malignant lymphoma, metastatic carcinoma, metastatic melanoma, metastatic sarcoma, etc. Afterwards, all the slides, corresponding to tissue block(s), and a corresponding pathology report can be submitted to expert pathologists for further consultation.

Careful study of clinical history of any possible causes of lymphadenopathy warrants the exclusion of mimics for malignancy of the lymph node, including drug reactions and immunodeficiency states.

A review of the clinical history is paramount in clinical practice. It helps in making a clinical impression of the most likely malignancy of the lymph node. Moreover, it

TABLE 5. Panel of immunostaining commonly used in diagnosis of malignant lymphomas and leukemia (after only CD45 expression in immunostaining panels proposed in Tables 3 or 4).[5]

Marker	Positive in	Remarks
T-cell & NK cell lymphoma		
CD3	Normal T-cells, T-cell lymphoma, T-LBL, and NK/T-cell lymphoma	Usually membrane staining but cytoplasmic staining in NK/T-cell lymphoma or T lymphoblast
CD2, CD5, CD7	Same as CD3	Common T-cell markers; aberrant loss of any of them raises concern of neoplastic nature
CD4 & CD8	Helper & cytotoxic T-cells	Normal ratio of 2:1 in peripheral lymphoid tissue and blood; double negative (CD4-CD8-) or double positive (CD4+ CD8+) phenotype deems neoplastic
TCR-beta (betaF1)	Normal T-cells	90% of peripheral blood T-cells
TCR-gamma (GTCR)	Normal T-cells & primary cutaneous gamma-delta T-cell lymphoma	10% of peripheral blood T-cells
EMA	Positive up to 85% of ALK+ ALCL	Epithelial cells, some plasma cells
PD1, CXCL13, CD10, BCL6, ICOS-1, HGAL	TFH	Need at least 2 markers positive for diagnosis of AITL or nodal peripheral T-cell lymphoma of TFH phenotype
CD21 & CD23	FDC meshwork & hyperplasia	FDC hyperplasia for AITL
CD30	Activated/transformed lymphoid cells, HRS cell & ALCL	Normal activated/transformed lymphoid cells in T-cell zone
ALK	ALK+ ALCL	Nuclear, nuclear + cytoplasmic, or cytoplasmic pattern
B-cell lymphoma & Plasma cell neoplasm		
CD20	Normal & neoplastic mature B-cells including NLPHL	Membranous staining; may be faint positive in SLL/CLL or negative in some B-cell neoplasms; negative in PCM but may be occasional positive
CD10	Germinal center B-cell (both reactive & neoplastic), B-LBL	Positive in normal marrow B-cell precursors, normal & neoplastic bile canaliculi (CC), normal & neoplastic renal tubule (RCC); endometrial stromal sarcoma; non-specific staining in myeloid series in the marrow
CD5	Normal T-cell & neoplastic B-cells in SLL/CLL & MCL	
CD23	Normal B-cell, FDC & neoplastic B-cells in SLL/CLL	CD5+ CD23+ in SLL/CLL but CD5+ CD23- cyclin D1+ in MCL; CD21 is an adjunct marker for FDC
Cyclin D1	MCL, PCM, HCL	Usually negative in small B-cells in normal mantle layer of lymphoid follicle; also positive in epithelial cells in cell cycle
SOX11	MCL	Negative in leukemic phase of MCL; not a sensitive marker in practice
CD38, CD138	Normal & neoplastic plasma cell	CD38 not equal to CD138 & both not equal to immunoglobulin staining; CD138 also positive in epithelial cell

TABLE 5. Panel of immunostaining commonly used in diagnosis of malignant lymphomas and leukemia (after only CD45 expression in immunostaining panels proposed in Tables 3 or 4).[5] (Continued)

Marker	Positive in	Remarks
B-cell lymphoma & Plasma cell neoplasm		
Kappa & lambda Ig light chains	Evaluation of Ig light chain expression in plasma cells	Normal kappa to lambda ratio of 2:1; when either kappa or lambda more than the other at least 5 times raises the concern of monoclonal plasma cell population (plasmacytic differentiation)
IgG, IgA, IgM	Evaluation of Ig heavy chain expression in plasma cells	Usually IgG > IgA > IgM; usually performed when kappa+lambda less than estimated number of plasma cells (by morphology or CD38/CD138 immunostaining) in order to determine heavy chain disease
MUM1	Normal & neoplastic plasma cells, subset of DLBCL	Large B-cell lymphoma with *IRF4* rearrangement (need FISH)
ALK	ALK+ large B-cell lymphoma	
Classic Hodgkin lymphoma		
CD3	T-cells	Negative in HRS cell
CD15	Neutrophil & HRS cell	Sometimes only paranuclear positivity in HRS cell; less sensitive in formalin-fixed tissue
CD20	B-cells & NLPHL	Negative in typical CHL but variably positive in occasional HRS cells is also accepted
CD30	Activated/transformed lymphoid cells, HRS cell & ALCL	Normal activated/transformed lymphoid cells in T-cell zone
CD45	All types of lymphoid cells & up to 50% of ALCL	Negative in HRS cell
EMA	Epithelial cells, some plasma cells	Negative in HRS cell
MUM1	Positive in HRS cell	Normal & neoplastic plasma cells
PAX5	B lymphoblast to mature B-cell stage; faint positive in HRS cell	Negative in plasma cell
Leukemic infiltration		
CD34	Hematopoietic stem cells, blasts, endothelial cells	Less sensitive for blasts than flow cytometry
TdT	B-LBL, T-LBL	
CD33, MPO	Myeloblast	AML, M0: CD33+ CD34+ but MPO-
CD14, CD68	Monocyte/promonocyte/monoblast	Immature morphology
CD99	Immature hematopoietic cell	Express on normal early thymocyte
CD117	Myeloid & erythroid precursors	Express on normal mast cells
CD123	BPDCN	Treated as ALL

Abbreviations: AITL: angioimmunoblastic T-cell lymphoma, ALCL: anaplastic large cell lymphoma, ALL: acute lymphoblastic leukemia, AML, M0: Minimally differentiated acute myeloid leukemia, B-LBL: B-lymphoblastic lymphoma/leukemia, BPDCN: blastic plasmacytoid dendritic cell neoplasm, CC: cholangiocarcinoma, FDC: follicular dendritic cell, FISH: fluorescence in situ hybridization, HCL: hairy cell leukemia, HRS: Hodgkin-Reed-Sternberg, Ig: immunoglobulin, MCL: mantle cell lymphoma, NLPHL: nodular lymphocyte predominant Hodgkin lymphoma, PCM: plasma cell myeloma (multiple myeloma), RCC: renal cell carcinoma, SLL/CLL: small lymphocytic lymphoma/chronic lymphocytic leukemia, TFH: T follicular helper, T-LBL: T-lymphoblastic lymphoma/leukemia

TABLE 6. Panel of immunostaining commonly used in diagnosis of carcinoma and epithelioid variant of sarcoma (after only AE1/AE3 expression in immunostaining panels proposed in Tables 3 or 4)[15,31]

Marker	Positive in	Remarks
Adenocarcinoma		
CK7 & CK20	CK7+/CK20+: Pancreas, bile duct, stomach, urinary bladder	CK7+/CK20-: Breast, endometrium, ovary, lung, thyroid (also positive in malignant mesothelioma)
	CK20+/CK7-: Colorectum, Merkel cell carcinoma & occasional upper GI	CK7-/CK20-: Adrenal cortical carcinoma, prostatic carcinoma, HCC, RCC, neuroendocrine carcinoma of lung & GI tract
CDX2	Colorectum	
GATA-3	Breast	Also positive in UC, pheochromocytoma, paraganglioma, choriocarcinoma, malignant mesothelioma
Hepar 1, Glypican-3, Arginase-1	HCC	
NKX3.1	Prostate, breast	Positive in invasive lobular carcinoma
PAX8	Endometrium, ovary, thyroid, RCC	PAX8+ CD45+ AE1/AE3- DLBCL
SATB2	Colorectum	Also positive in neuroendocrine carcinoma, osteosarcoma, BCOR-rearranged sarcoma
TTF-1	Lung, thyroid	
Squamous cell carcinoma		
CK5/6	+	Also positive in mesothelioma, BCC, UC
p40	+	Also positive in BCC, UC
p63	+	p63+ CD45+ AE1/AE3- DLBCL
Neuroendocrine carcinoma		
CD56	+	Also + in other types of cancer
CK8/18	+	
Chromogranin A	+	
Synaptophysin	+	
Ki-67	>20%	<20% in neuroendocrine tumor
Epithelioid variant of sarcomas		
CD34, ERG	Epithelioid Angiosarcoma	Loss of SDH subunit B
CD117, DOG1	SDH-deficient GIST	
Desmin, myogenin	Epithelioid RMS	
INI-1 loss	ES, epithelioid MPNST	Also positive in melanoma & LCH
S100	Epithelioid MPNST	Also positive in melanoma
SOX10	Clear cell sarcoma	Also positive in ES, ESS, EWS, MPNST, schwannoma, and SFT
TLE-1	Synovial sarcoma	

Abbreviations: BCC: basal cell carcinoma, DLBCL: Diffuse large B-cell lymphoma, ES: epithelioid sarcoma, ESS: endometrial stromal sarcoma, EWS: Ewing sarcoma, HCC: hepatocellular carcinoma, LCH: Langerhans cell histiocytosis, MPNST: malignant peripheral nerve sheath tumor, RCC: renal cell carcinoma, RMS: rhabdomyosarcoma, SCC: squamous cell carcinoma, SDH-deficient GIST: succinate dehydrogenase-deficient gastrointestinal tumor, UC: Urothelial carcinoma

TABLE 7. Panel of immunostaining commonly used in diagnosis of malignant melanoma and sarcoma (after only S100 expression in immunostaining panels proposed in Tables 3 or 4)[31]

Marker	Positive in	Remarks
Malignant melanoma		
SOX10		Also positive in clear cell sarcoma & epithelioid MPNST
HMB45		Also positive in angiomyolipoma
Melan A		Also positive in adrenal cortical carcinoma & stromal sex cord tumor
Sarcoma		
See epithelioid variant of sarcoma in Table 6		

Abbreviation: MPNST: malignant peripheral nerve sheath tumor

helps to exclude reactive conditions, infectious processes, and abnormal immune reactions, especially drug reactions and immunodeficiency states, as discussed earlier. The location of the enlarged lymph node implies possible causes such as metastatic CA breast in the axillary lymph node in female patients, metastatic nasopharyngeal carcinoma in level II (upper jugular group) of cervical lymph nodes, metastatic CA thyroid in level IVa (lower jugular group), IVb (medial supraclavicular group), or VIb (deeper pre-laryngeal/pre-tracheal group) of cervical lymph nodes, metastatic CA stomach in left supraclavicular lymph node, and metastatic melanoma in inguinal lymph node. However, at times, unexpected metastatic tumors are observed in unusual locations such as the CA prostate with metastasis to the cervical lymph node.[25] However, the most difficult case is cancer of unknown primary (CUP) in clinical practice. CUP rarely presents in lymph node only (LNCUP), according to a series from MD Anderson Cancer Center. LNCUP has better clinical outcomes than CUP in general or CUP with predominant bone disease.[26] While axillary LNCUP in women is treated as CA breast with axillary lymph node metastasis, histologic type of adenocarcinoma seems to have better clinical outcomes than other histologic types.[27] In a LNCUP case with only one lymph node group positive for metastatic carcinoma, it may be possible to track the primary site according to sentinel node theory, but it is limited to one organ with one direction of lymphatic drainage. It cannot be applied to supraclavicular lymph nodes that receive lymphatic drainage from many organs or lymph node metastasis with more than two directions of lymphatic drainage.[28]

Adequate cell or tissue samples will allow pathologists to work efficiently and master the multimodality approach under good clinical collaboration.

General practitioners, especially ones who perform tissue biopsy, can help by paying attention to the details of the tissue obtained. Most cellular tumors have soft to firm light brown tissue texture with or without accompanying hemorrhaging or necrosis. White tough fibrous tissue should be avoided and if the tissue biopsy looks like that, doing another biopsy to obtain more representative tissues is a must or the pathology report will come back as "fibrotic tissue obtained, please do another biopsy." In the past, pathologists preferred complete excision of the enlarged lymph node so that a complete evaluation could be performed. Fine needle aspiration (FNA) of the lymph node is not recommended in suspected cases of malignant lymphoma. However, technical advances have provided options for improvements such as a FNA accompanied by flow cytometry for lymphoma panel. However, core needle biopsy is widely accepted in practice but a large panel of immunostaining is required to obtain more information to compensate the limited histologic evaluation.

Effective communication between pathologists and physicians regarding relevant laboratory investigations should make it easier to make a definitive diagnosis for the type of malignancy

There is no doubt that effective communication between pathologists and physicians is the best way to achieve a definitive diagnosis of a malignancy based

on relevant laboratory investigations in addition to good clinical history as mentioned earlier. Even better access via a laboratory information system (LIS) allows pathologists to find relevant laboratory results easier, while a discussion of the case with attending physicians usually reveals important issues of concern about the diagnosis of tumor type. However, in a small number of cases, it is very difficult to acquire a definitive definite diagnosis when the tumor cells do not differentiate well (undifferentiated tumor).

Pathology of tissue sample handling and diagnostic process

The way pathologists handle cell or tissue samples by conventional morphologic evaluation and panels of immunostaining provides insight to general practitioners to understand the diagnostic process in pathology. In terms of quality assurance, laboratory work involves pre-analytic, analytic, and post-analytic phases. The pre-analytic phase focuses on specimen collection. In this step, the general practitioner needs to know how to obtain the specimen correctly and choose the appropriate test. Regarding pathologic diagnosis, FNA, core needle biopsy, incisional biopsy or excision of the enlarged lymph node should be selected based on clinical information as well as accessibility to the lesion of concern. FNA of the cervical lymph node with a clinical concern of nasopharyngeal carcinoma or cancer of the head and neck region is deemed appropriate. In contrast, this process is not rewarding for malignant lymphoma unless a flow cytometry of fresh samples from FNA is performed at the same time. So, FNA is not recommended in a suspected case of malignant lymphoma where flow cytometry is not available. Instead, excision of the enlarged lymph node is recommended. In places with limited resources, imprints of a fresh cut surface of the lymph node provide cytologic features of lymphoma cells. A good cytotechnologist or even a well-trained hematologist can make a definitive diagnosis from well prepared lymph node imprints, such as Burkitt lymphoma.

Another important issue in the pre-analytics phase is the quality of tissue sample. It should be handled properly so that the slides stained with hematoxylin and eosin (H&E) can be examined by pathologists without interference in the interpretation process. The tissue sample should be fixed in a good volume of neutral buffered formalin (10 times the volume of the tissue sample) for at least two to three hours prior to tissue processing in the laboratory. If core needle biopsy of the lymph node is performed, practitioners must make sure that a proper lymph node tissue is obtained. The tissue should be soft to firm and light brown. If the tissue is tough white fibrous or pale yellow necrotic, more tissue cores are needed. In case of necrotic tissue, microbiologic studies should be considered as well.

The analytics phase depends on a pathologist's performance. Good quality H&E-stained slides should allow pathologists to gather relevant microscopic findings to make a diagnosis. Recognition of particular patterns should lead to a list of differential diagnosis. Ultimately, it depends on a pathologist's knowledge of histology, pathology, training background, technical skills, perception, and memory.[29] Then, all clinical and pathological findings are analyzed using the pathologist's knowledge and experience to provide an interpretation of the lymph node biopsy. The diagnosis could be a straightforward textbook case for any malignancy of the lymph node, however, in a problematic case, additional clinical and laboratory information as well as clinical impression must be considered. Special stains, histochemistry, and immunostaining are requested in order to gather more information to decide the nature of tumor cells. At this point, authorization and expenses can take time, depending on the health care system. If the chain of analysis is allowed to flow freely, an experienced pathologist may handle the case efficiently and provide a diagnosis within 24 or 48 hours, based on histologic evaluation, immunostaining, and/or a number of in situ hybridization (ISH) techniques such as ISH for EBV-encoded small RNA (EBER ISH). In some institutes, a double sign-out system will ask two pathologists to look at the case of malignancy diagnosed for the first time in order to confirm a diagnosis before releasing the pathology report.[30]

The post-analytic phase depends on the attending physician determining whether the pathologic diagnosis of any type of the malignancy of the lymph node is clinically relevant. In some institutes, before releasing the pathology report, the pathologist and the physician who submits the tissue sample have already discussed the case. Any physician who later gets involved in patient management has a right to challenge the diagnosis when it is not relevant to the clinical information. For example, the pathologist gives a diagnosis of CHL but there are a number of clinical findings suggest NHL. The pathologist should be notified and asked to review the case. Moreover, in any malignancy diagnosed from other hospitals, all pathologic materials (slides, corresponding tissue blocks, and pathology report) should be reviewed by an experienced pathologist to confirm the diagnosis before starting any specific treatment.

CONCLUSION

By understanding the basic principles and the types of malignancy found in the lymph node, a careful study of the clinical history, adequate cell or tissue samples from the lymph node, good clinical collaboration with effective communication between pathologists and physicians, and mastery in conventional morphologic evaluation along with an appropriate panel of immunostaining, general practitioners and pathologists could make the diagnostic process easier in order to diagnose the malignancy of the lymph node in most cases.

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