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Contents

KMJ
KOSIN MEDICAL JOURNAL

Vol. 37, No. 1 March 2022

Editorial

1 Is ketamine useful for pain management in patients with stage IV cancer?
Sung Eun Kim

Review articles

4 Role of biomarkers in the heart failure clinic
Bong-Joon Kim, Jae-Hyeong Park

18 Adverse events following vaccination against coronavirus disease 2019
Minji Jeon, Sol Jin, Jin-Young Lee

27 Sleep and vaccine administration time as factors influencing vaccine immunogenicity
Eun Seok Kim, Chi Eun Oh

Original articles

37 The effects of ketamine on pain control in stage IV cancer patients receiving palliative care
Seonghoon Kim, Jihun Kang, Jongsoon Choi, Eunhee Kong

46 Comparison of the radiation dose between dual-acquisition coronary computed tomography angiography and coronary angiography for coronary spasm
Soo-Jin Kim, Moo Hyun Kim, Eun-Ju Kang

52 Transcriptome analysis of the pathogenic ciliate *Miamiensis avidus* after hydrogen peroxide treatment
Hyunsu Kim, A-Reum Lee, Kyung-Yoon Jeon, Eun-Ji Ko, Hee-Jae Cha, Mee Sun Ock

61 Validation of the association of the cystic duct fibrosis score with surgical difficulty in laparoscopic cholecystectomy
Hyung Hwan Moon, Ji Hoon Jo, Young Il Choi, Dong Hoon Shin

68 Hepatic steatosis changes after early gastric cancer surgery
Ki Hyun Kim, Soyoung Ock, Dohyung Lee, Yoonhong Kim, Jihoon Jo, Kyungwon Seo, Kiyoung Yoon, Sukyoung Kwon, Youngsik Choi, Bukyung Kim

75 New approach to learning medical procedures using a smartphone and the Moodle platform to facilitate assessments and written feedback
Sang-Shin Lee, Haeyoung Lee, Hyunyong Hwang

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Contents

KMJ
KOSIN MEDICAL JOURNAL

Vol. 37, No. 1 March 2022

Case reports

83 Metastatic ameloblastoma with postoperative accelerated tumor growth treated with carboplatin and paclitaxel: a case report

Joo Un Park, Jae Wan Jung

89 Emergency rescue stent grafting for a ruptured degenerated graft that bypassed coarctation of the aorta: a case report

Jin Ho Choi, Min Suk Choi, Haeyoung Lee

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Editorial

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Is ketamine useful for pain management in patients with stage IV cancer?

Sung Eun Kim

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See "The effects of ketamine on pain control in stage IV cancer patients receiving palliative care" by Seonghoon Kim, Jihun Kang, Jongsoon Choi, Eunhee Kong.

Pain is a debilitating symptom of cancer, and its incidence is estimated to be greater than 90% in the advanced stage of the disease. According to the voluminous systematic review of 52 studies over 40 years, the pooled prevalence rate of cancer pain in all stages of disease was 53% (confidence interval [CI], 43%–63%), and those of cancer pain in advanced, metastatic, or terminal disease was 64% (CI, 58%–69%) [1]. More than one-third of cancer patients with pain revealed the pain intensity to be moderate or severe [1]. Therefore, several guidelines for the management of cancer pain have been published by the World Health Organization, National Comprehensive Cancer Network, American Society of Clinical Oncology, and European Society of Medical Oncology (ESMO). However, cancer pain remains an important subject for physicians in the clinical field.

Cancer pain has varied management depending on the severity of pain. Nonsteroidal anti-inflammatory drugs and paracetamol are generally recommended for the management of mild cancer pain, and opioids are the cornerstone of analgesic therapy for managing moderate to severe cancer pain. In addition, non-opioid analgesics such as ketamine and lidocaine have also been suggested as adjuncts for the management of patients with cancer pain [2].

Ketamine, a non-competitive N-methyl D-aspartate (NMDA) receptor antagonist, is a phencyclidine intravenous anesthetic used to induce and maintain general anesthesia due to its glutamate blocking effect. Interestingly, low (sub-anesthetic) doses of ketamine have analgesic effects and may suppress central sensitization, hyperalgesia, and opioid tolerance, which might be associated with neuropathic pain [2]. The reason for these effects is presumed to be that the ketamine may inhibit the activation of NMDA receptors in secondary afferent neurons, which is considered indispensable for enhancing pain sensitivity by repetitive nociception stimuli [3]. Unfortunately, there are only a few studies evaluating the use of ketamine as an adjuvant to opioids for the management of cancer pain, and the ESMO guidelines reported that there is a lack of evidence to support the routine use of ketamine in neuropathic cancer pain [4]. The effects of ketamine on pain control in patients with cancer are still controversial.

In this issue of *Kosin Medical Journal*, Kim et al. [5] investigated the effects of intravenous ketamine in patients with stage IV cancer with pain. A total of 253 patients were enrolled retrospectively, of which 112 patients received ketamine (ketamine group) and 141 patients did not receive

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ketamine (control group). The primary outcome identified a change in pain scores in both groups between the time of ketamine introduction and after 48 hours of introduction, and included proportions of a favorable response (pain score declined by ≥2), optimal response (pain score declined by ≥50%), and opioid-sparing effect (a reduction of opioid dose due to ketamine). The secondary outcome verified a decrease in visual analog scale (VAS), oral morphine equivalents (OME), inter-dose opioid frequency, and inter-dose opioid amounts in the ketamine and control groups at the time of ketamine administration, 24 hours, and 48 hours after administration. Based on the study results, the proportions of favorable response (odds ratio [OR], 3.84; 95% CI, 1.76–8.37) and optimal response (OR, 3.99; 95% CI, 1.73–9.22) in the ketamine group were higher than those in the control group. The degree of VAS score decreased more in the ketamine group than in the control group both at 24 hours and 48 hours after ketamine administration. In terms of adverse events, the ketamine group had a lower risk of depressive mood (OR, 0.31; 95% CI, 0.10–0.92) and a higher risk of delirium (OR, 2.06; 95% CI, 1.12–3.91) compared to the control group.

A recent retrospective study from Hong Kong showed that a favorable response rate to ketamine was 74.3% (n=52) in patients with cancer with refractory pain [6]. Another systematic review also reported that ketamine may be a feasible option for treatment of refractory cancer pain, despite data limitations [7]. However, a recent Cochrane review determined the effectiveness and adverse effects of ketamine as an adjuvant to opioids for refractory cancer pain in adult patients [8]. The Cochrane review included a total of three studies. Two studies showed that adding ketamine to morphine reduced pain severity and morphine requirements; however, one study that administered high-dose ketamine to opioids did not show any clinical benefit. Therefore, the review concluded that there was insufficient evidence to assess the effectiveness and adverse effects of ketamine as an adjuvant to opioids [8].

This study had several limitations. Firstly, the ketamine group consisted of patients with refractory cancer pain without adequate response to the administration of rapid-active morphine (20% of regular opioid dose) and increasing the dose of regular opioids (50%–100% increase of previous regular opioid dose); while the control group was defined as non-ketamine users. Therefore, selective bias cannot be ignored in this study design. Similarly, the initial VAS scores, OME, inter-dose opioid frequency, and inter-dose opioid amounts varied between the ketamine and control group. Second, since the initial VAS score of the control group was <3, it is estimated that it might be difficult to achieve a favorable or optimal response compared to the ketamine group with an initial score >3, which may have influenced the results. Finally, although this was a retrospective study, the effectiveness and adverse effects of ketamine could have been better identified if placebo was also administered in the ketamine group.

Nonetheless, to date, studies investigating the effectiveness of ketamine in the management of cancer pain are few in number, and few studies have been conducted among adult stage IV cancer patients with cancer pain. To our knowledge, this is the first study to determine the effectiveness of ketamine in the management of cancer pain in Korean patients with stage IV cancer receiving palliative care. Further large-scale prospective studies are required to understand the effectiveness and pathophysiology of ketamine for the management of cancer pain in patients with cancer with moderate to severe pain.

Article information

Conflicts of interest

Sung Eun Kim is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Review article

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Role of biomarkers in the heart failure clinic

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Heart failure (HF) is a common cardiovascular disease that has a complex pathophysiology. Because it is the final stage of many cardiovascular diseases, proper diagnosis and treatment are crucial for prolonging patients' survival and improving their well-being. Several biomarkers have been identified in HF, and their roles in diagnosis and prognostication have been widely investigated. Among them, natriuretic peptides are key for diagnosing HF, predicting its prognosis, and monitoring the effectiveness of HF treatment. Moreover, natriuretic peptides can also be used to treat HF. In addition to natriuretic peptides, several other biomarkers were included in the most recent HF management guidelines. Thus, we reviewed the role of the biomarkers included in these guidelines and discussed future perspectives.

Keywords: Biomarker; Galectin-3; Natriuretic peptides; Soluble ST2; Troponin

Introduction

Heart failure (HF) is a complex cardiovascular disease with multiple pathophysiologic mechanisms, so it is considered the final stage of many cardiovascular diseases. The prevalence of HF continues to increase over time due to the aging and the increase of comorbid cardiovascular diseases. The estimated prevalence of HF is 1% to 2% of the general adult population [1], and approximately 6,000,000 American adults over the age of 20 have experienced HF according to US data between 2015 and 2018 [2]. Prompt diagnosis and treatment of HF can reduce its associated socioeconomic burden. However, the diagnosis of HF usually relies on symptoms, physical examination, serum concentration of natriuretic peptides, and left ventricular systolic and diastolic function assessed by echocardiography. Recent treatment guidelines for HF include natriuretic peptides in support of the diagnosis, prediction of prognosis, and management of HF patients. As the coronavirus disease 2019 (COVID-19) pandemic has expanded, physical examination has become challenging, with difficulties to diagnose HF in patients presenting with dyspnea and chest discomfort. Therefore, the importance of biomarkers has increased, especially in patients with restricted physical examinations [3]. Besides natriuretic peptides, several other biomarkers have been introduced to support the understanding of the pathophysiology of HF, improving personal care through better individual HF phenotyping [4].

In this review article, we will discuss the current use of biomarkers in the diagnosis and management of HF and their future perspectives.

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Biomarkers of the pathophysiology of heart failure

There are many biomarkers involved in the pathophysiological mechanisms of HF, which can be classified into four categories: myocardial damage, neurohormonal activation, myocardial remodeling, and biomarkers of inflammation and oxidative stress (Table 1). Although there are many biomarkers involved in the development of HF, only a few are currently available in clinical practice. B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) not only play an important pathophysiological role in the HF development, but also increase as the disease progresses, so they can be used for the diagnosis or monitoring of HF. BNP and NT-proBNP are major biomarkers widely used in the HF clinic because they can reflect the clinical status of patients aiding clinical judgment for the diagnosis, management, and prognosis of HF.

Biomarkers recommended in recent heart failure treatment guidelines

The 2021 European Society of Cardiology (ESC) guidelines for HF have been updated to reflect recent clinical studies, and notable changes have been made in HF management [5]. These focus on BNP/NT-proBNP as biomarkers for diagnosis and treatment of HF [5], emphasizing their role in the diagnostic process of chronic HF, acute HF, HF with preserved ejection fraction (HFpEF), and advanced HF. The role of most other biomarkers has not been highlighted; only troponin is recommended to rule out acute coronary syndrome (ACS) in the acute HF setting (Table 2).

The 2017 ACC/AHA/HFSA focused update guideline for the management of HF also emphasized BNP/NT-proBNP for HF prevention, diagnosis, and prognosis (as added risk stratification) [6]. In these treatment guidelines, biomarkers other than natriuretic peptides are mentioned. Biomarkers such as soluble suppression of tumorigenicity 2 (sST2) and galectin-3 are acknowledged to predict death and hospitalization and also provide prognostic value over natriuretic peptide levels for HF patients as class IIb recommendations (Table 3).

Although the 2021 ESC guideline is the most recently updated, the fact that it does not include the biomarkers mentioned in the 2017 American College of Cardiology guideline suggests that there is little clinical evidence in their support.

BNP and NT-proBNP

1. Synthesis and excretion of BNP/NT-proBNP

BNP and NT-proBNP are biomarkers indicating myocardial stretch, it has sufficient evidence for HF diagnosis [4]. *BNP* gene expression increases as a result of myocardial ischemia or myocardial stretch [7]. BNP is synthesized as proBNP with 108 amino acids in cardiomyocytes. When proBNP is released into the circulation, it is cleaved into inactive NT-proBNP with 76 amino acids and active BNP with 32 amino acids. BNP has various biological effects, including vasodilatation, natriuresis and inhibition of the renin-angiotensin-aldosterone system and sympathetic nervous system

Table 1. Biomarkers of pathophysiologic pathways contributing to heart failure development

Mechanism	Biomarkers
Myocyte stretch	Atrial natriuretic peptide (ANP), mid-regional proANP
	B-type natriuretic peptide (BNP), N-terminal proBNP
	Growth differentiation factor (GDF)
	Neuregulin
	Soluble suppression of tumorigenicity 2 (sST2)
Neurohumoral activation	Norepinephrine
	Renin
	Angiotensin II
	Aldosterone
	Arginine vasopressin
	Endothelin-1
	Chromogranin A and B
	Adrenomedullin
Myocardial damage	Cardiac troponins (TnT, TnI, and hsTn)
	Creatinine kinase-MB (CK-MB)
	Heart-type fatty acid-binding protein
	Soluble Fas cell surface death receptor (sFAS)
	Heat shock protein 60
	Soluble TNF-related apoptosis-inducing ligand (sTRAIL)
	Pentraxin 3
Biomarkers of comorbidity	Inflammation:
	C-reactive protein (CRP), tumor necrosis factor-α (TNF-α), lipoprotein-associated phospholipase A2 (LP-PLA2), IL-1, IL-6, IL-10, IL-18
	Adipokines, polacinonin, cytokines
	Oxidative stress:
	Myeloperoxidase, oxidized low-density lipoproteins, plasma malondialdehyde

Table 2. Biomarkers for heart failure recommended by the European Society of Cardiology 2021 guidelines

Clinical setting	Biomarker	Class of recommendation	Level of evidence	Reference value
Chronic HF				
Diagnostic test for suspected chronic HF	BNP, NT-proBNP	I	B	NT-proBNP ≥125 pg/mL BNP ≥35 pg/mL
To rule out HF	MR-proANP	None	None	<40 pmol/L
Objective evidence of serologic abnormalities in HFpEF	BNP/NT-proBNP	None	None	NT-proBNP >125 pg/mL (SR) or 365 pg/mL (AF) BNP >35 pg/mL (SR) or 105 pg/mL (AF)
Advanced HF				
Criteria for advanced HF	BNP/NT-proBNP	None	None	Persistently high (or increasing) BNP or NT-proBNP value
Acute HF				
Diagnostic test for acute HF (to rule out AHF)	BNP/NT-proBNP, MR-proANP	IIa	None	BNP ≥100 pg/mL, NT-proBNP ≥300 pg/mL[a], MR-proANP ≥120 pg/mL
To exclude ACS	Troponin	I	None	None

HF, heart failure; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal proBNP; MR-proANP, mid-regional pro-atrial natriuretic peptide; HFpEF, heart failure with preserved ejection fraction; SR, sinus rhythm; AF, atrial fibrillation; AHF, acute heart failure; ACS, acute coronary syndrome.
[a]Rule-in values for the diagnosis of acute HF: >450 pg/mL for age <55 years, >900 pg/mL for 55–75 years, and >1,800 pg/mL for age >75 years.

Table 3. Biomarker indications according to 2017 ACC/AHA/HFSA focused update [6]

Clinical setting	Biomarkers	Class of recommendation	Level of evidence	Comments
HF prevention				
Patients at risk of developing HF	BNP, NT-proBNP	IIa	B	Can be useful to prevent LV dysfunction (systolic or diastolic) or new-onset HF
HF diagnosis				
Patients with dyspnea	BNP, NT-proBNP	I	A	Useful to support diagnosis or exclude HF
Prognosis of added risk stratification				
Chronic HF	BNP, NT-proBNP	I	A	Useful for establishing prognosis or disease severity
Baseline measurement at hospital admission	BNP, NT-proBNP, and cardiac troponin	I	A	Useful to establish prognosis in acutely decompensated HF
During HF hospitalization, pre-discharge measurement	BNP, NT-proBNP	IIa	B	Can be useful to establish post-discharge prognosis
Chronic HF	Biomarkers of myocardial injury or fibrosis (soluble ST2, galectin-3, hs-cardiac troponin, and others)	IIb	B	Predictive of hospitalization and death in HF patients and also additive to NP levels[a]

ACC/AHA/HFSA, American College of Cardiology/American Heart Association/Heart Failure Society of America; HF, heart failure; BNP, B-type natriuretic peptide; NT-proBNP, N-terminal proBNP; LV, left ventricle; ST2, suppression of tumorigenicity 2; NP, natriuretic peptide.
[a]A combination of biomarkers may be more informative than single-biomarker measurements.

through interaction with natriuretic peptide receptor type A, resulting in increased production of intracellular cyclic guanosine monophosphate. BNP binds to the natriuretic peptide type C and is eliminated through proteolysis by neutral endopeptidase, with a half-life of approximately 20 minutes. And NT-proBNP, the inactive form, is mainly cleared by renal excretion, and its half-life is approximately 120 minutes.

2. Clinical relevance of BNP and NT-proBNP

After BNP was discovered to be secreted during myocardial stretch and to have favorable effects such as natriuresis, it was initially used for the treatment of HF patients [8]. Yoshimura et al. [8] reported that infusion of synthetic human BNP improves left ventricular function in heart failure reduced ejection fraction (HFrEF) through natriuretic effects and vasodilation. However, BNP and NT-proBNP were

found to be related to symptoms assessed by the New York Heart Association, the functional class, hemodynamic status in the setting of volume expansion or pressure overload, and left ventricle (LV) diastolic and systolic function [9,10]. Thus, these have been widely used to diagnose and monitor HF.

According to a randomized controlled trial, amino-terminal proBNP testing significantly improved the accuracy of HF diagnosis in the general population [11], and other studies support the use of BNP/NT-proBNP testing in primary care to evaluate HF in non-acute settings [12-14]. In an acute HF setting, the NT-proBNP level is the standard clinical assessment with a very high negative predictive value to identify or rule out acute HF [15]. A meta-analysis acknowledged that testing the NT-proBNP level in suspected acute HF patients enables prompt and precise exclusion of the diagnosis [16]. Especially for patients who require a rapid evaluation for potential HF and who cannot undergo echocardiography in an emergency department, BNP and NT-proBNP testing can provide meaningful information or differential diagnosis of HF. And it is highly probable that the symptoms and signs of a patient with normal BNP or NT-proBNP are not related to HF, and further investigation should be performed to identify other causes of these signs and symptoms [17].

In addition, natriuretic peptides can be useful not only for diagnosing HF but also for predicting prognosis. Therefore, to measure the BNP or NT-proBNP level is clinically relevant to estimate not only the presence but also the severity of HF, indicating clinical improvement in response to medical treatment [18-20]. Several clinical trials have used natriuretic peptide levels to evaluate the effectiveness of medical treatment and indicate clinical improvement, and some have shown that biomarker-guided treatment can improve the outcomes of HF [21,22]. In the STOP-HF (St Vincent's Screening To Prevent Heart Failure) study, treatment with BNP-based screening and collaboration reduced the rates of events of HF and LV systolic dysfunction, diastolic dysfunction [23]. According to a study that compared the prognostic value of NT-proBNP in advanced HF with that of other parameters, NT-proBNP measurement alone was a better prognostic meaning than LV ejection fraction (LVEF), peak VO_2, and HF survival score [24].

Another important point is that BNP/NT-proBNP have been used worldwide for a long time and have recognized cutoff values. In the non-acute setting, the upper normal limits are 35 pg/mL for BNP and 125 pg/mL for NT-proBNP [25-27].

Various studies have reported that natriuretic peptide levels can be used as a parameter of clinical improvement in response to medical care. However, the associated prognoses have been inconsistent. The TIME-CHF trial (Trial of Intensified vs. Standard Medical Therapy in Elderly Patients With Congestive Heart Failure) showed that HF therapy guided by NT-proBNP did not enhance composite clinical outcomes or quality of life than symptom-guided treatment [28]. Also, in the GUIDE-IT study (Guiding Evidence Based Therapy Using Biomarker Intensified Treatment in Heart Failure), treatment with NT-proBNP-guide did not improve first HF hospitalization or cardiovascular death [29]. Consistent evaluation is difficult due to differences in the target patient groups and study designs; however, it does appear that natriuretic peptide-guided treatment does not always show better outcomes than symptom-guided treatment. Since GUIDE-IT is a large-scale, multicenter, randomized trial, its results should not be overlooked.

3. Limitations of using BNP and NT-proBNP

Although structural cardiac and non-cardiac diseases are associated with increased BNP/NT-proBNP levels, a single measurement can reduce the confidence of HF diagnosis. Clinicians should be aware of the caveats of testing. First, elevated BNP or NT-proBNP levels could be due to various causes, including non-cardiac factors, which can reduce diagnostic accuracy [5]. However, levels are usually lower than those in acute decompensated HF patients [4]. Also, BNP or NT-proBNP levels can increase with age. The ESC practical guidelines for natriuretic peptide concentrations suggest different cutoff values for acute and chronic settings and age groups [9]. Age in particular increases the cutoff value of the gray zone and increases the need for cautious interpretation. Second, chronic kidney disease patients usually have higher BNP or NT-proBNP levels. Accumulation of these natriuretic peptides in chronic kidney disease patients occurs because of their decreased secretion accompanied by an increased release of these peptides due to hypertension and chronic volume overload comorbidities. Thus, the cutoffs of BNP at 200 pg/mL and NT-proBNP at 1,200 pg/mL provide an appropriate diagnostic performance in patients with renal insufficiency defined as decreased estimated glomerular filtration rate <60 mL/min per 1.73 m^2

[4]. Third, obesity is known to be associated with lower BNP levels than traditional cutoff values used in the diagnosis of HF due to a variety of physiological and metabolic mechanisms [30,31]. Fourth, low BNP concentration may also be detected in patients with advanced end-stage HF or right HF [9]. Therefore, the BNP levels of patients with relatively stable HF should be interpreted with care [32]. Fifth, among others, lung disease and atrial arrhythmias can also affect the BNP and NT-proBNP levels, so their interpretation must take these factors into consideration [9]. These particular comorbidities are very common in HF patients, and may limit interpreting the value of BNP and NT-proBNP levels in clinical practice.

Mid-regional pro-atrial natriuretic peptide

Atrial natriuretic peptide (ANP) is produced in the atrium in response to increased wall stress secondary to intravascular volume increase [33,34]. Mid-regional pro-ANP (MR-proANP) is another biomarker of myocardial remodeling, and its diagnostic role has been established [35]. The serum level of MR-proANP is not influenced by anemia and obesity [35,36]. The 2021 ESC guidelines suggest the use of MR-proANP to rule out HF in the diagnosis of new-onset acute HF. In a large, multicenter, prospective, trial of patients presenting to an emergency department with dyspnea, MR-proANP showed that it was not inferior to BNP for the diagnosis of acute HF [37]. Another study also showed that MR-proANP had a value comparable to that of NT-proBNP in the diagnosis of acute HF [38]. Heining et al. [39] showed that MR-proANP achieved an AUC of 0.83 in the diagnosis of acute HF. Their study applied a cutoff value of 120 pmol/L, produced a sensitivity of 91.1% and a negative predictive value of 92.1%.

However, increased MR-proANP levels have been found in conditions such as atrial arrhythmia [40,41], sepsis, respiratory tract infections, ventilator-associated pneumonia [42,43], and renal dysfunction [44]. And most studies using MR-proANP have focused on the acute HF setting, only few studies have looked at the early stages of HF. In addition to most of these considered the prognostic (not diagnostic) role of MR-proANP. Therefore, its diagnostic use in patients with symptoms suggestive of HF has not been established in non-acute HF settings [45]. More research is needed in the future.

Cardiac biomarkers other than natriuretic peptides

1. Cardiac troponins

Cardiac troponins are biomarkers indicating myocardial damage and their measurement is the gold standard to diagnose acute myocardial infarction [46]. In the updated 2021 ESC guidelines, cardiac troponin is recognized as having a limited role. Among laboratory tests, cardiac troponins are useful for detecting ACS, although elevated levels are detected in the majority of patients with acute HF [5]. Troponins can be elevated for a variety of reasons, including renal failure, stroke, pulmonary thromboembolism, sepsis, cardiac causes such as cardiac surgery, cardioversion, LV hypertrophy, and arrhythmia, all of which can increase the risk of HF [47]. However, according to a previous review article, about 20% of acutely symptomatic patients admitted to an emergency room had elevated levels of cardiac troponins, and most of them did not have ACS [48]. A post-hoc analysis of another cohort reported that high-sensitivity (hs)-troponin I was elevated in the vast majority of hospitalized patients and more than 50% of outpatients with HFpEF [49]. The usefulness of troponin seems to be underestimated not only in the HF guidelines, but also in clinical practice.

Troponin T and troponin I release can occur in patients with HF in the absence of an ACS event [50]. The mechanism of troponin elevation is explained by myocardial oxygen demand-supply mismatch and abnormal microvascular growth patterns [51]. Other mechanisms leading to increased troponin level in HF remain elusive in many cases, but they prominently include supply–demand inequity, associated with coronary artery obstruction and endothelial dysfunction, anemia, or subendocardial injury [52]. Sato et al. [53] showed that persistently increased troponin concentrations in dilated cardiomyopathy suggest ongoing subclinical myocyte degeneration, which is associated with deterioration of patient clinical status.

Cardiac troponins were found to be helpful in diagnosing both HF and ACS in the large-cohort ADHERE study (Acute Decompensated Heart Failure National Registry). Troponins are good predictors of short-term in-hospital mortality in acute decompensated HF patients, and cardiac troponin I and T have identical predictive value [54]. In both

acute HF and chronic stable HF, troponin is a significant prognostic factor. Tentzeris et al. [55] evaluated the complementary role of copeptin and cardiac troponin T in the identification of high-risk chronic HF, and the combination of hs-troponin T and copeptin may predict clinical outcome. Also, troponins are useful surrogate markers in chemotherapy-induced cardiomyopathy. A cardio-oncology working group proposed using periodic troponin measurements to detect chemotherapy-induced cardiotoxicity [56]. In addition, measuring changes in troponin levels helps predict the prognosis of HF [52].

Most hospitals use either troponin I or troponin T, but there is no evidence that either is superior. In the general population, troponin I and T levels show some statistical differences in predicting CVD, but both are significant indicators for HF prediction [57]. However, most studies using troponin have used retrospective designs, and many of them did not completely exclude factors that could affect troponin levels.

2. Soluble ST2

ST2 is a member of the interleukin (IL)-1 receptor family with transmembrane and soluble isoforms (soluble ST2, sST2), and is a biomechanically-derived protein synthesized primarily by cardiac fibroblasts. IL-33-ST2 signaling plays an important role in the mechanistically-activated cardioprotective fibroblast–myocardial paracrine system. Thus, IL-33 may have therapeutic potential for treating the myocardial response to overload. sST2, a biomarker of myocardial stretch, blocks the anti-hypertrophic effects of IL-33, and measuring sST2 provides useful information as a biomarker for HF [58].

The clinical trials measuring sST2 in patients with HF are summarized in Table 4. sST2 has been shown to discriminate HF and predict its prognosis in various clinical settings, acute and chronic HF in particular. A previous meta-analysis of sST2 has shown that it has prognostic value in predicting composite outcomes in acute HF [59]. In a relatively large-cohort study (HF-ACTION), sST2 was independently associated with clinical outcomes after adjusting for NT-proBNP in the multivariable models, and higher sST2 was associated with both poor functional capacity and poor prognosis [60]. Another study reported that sST2 was associated with cardiac abnormalities prevalent in echocardiography, including increased LV end-systolic dimension/volume and decreased LVEF [61]. In a head-to-head comparison (PROTECT trial) of serial sST2, hs-troponin T measurements and growth differentiation factor-15, only sST2 approved to add prognostic information to the baseline levels and predict changes in LV function in a chronic HF setting [62].

Unlike natriuretic peptides, sST2 is not significantly related to age, heart rhythm, or body mass index (BMI) [63]. The relative independence of sST2 from common comorbidities is a potential advantage. In terms of biological variability, sST2 is a good biomarker for HF. Piper et al. [64] examined patients with HF to determine the biological variability of sST2 by collecting blood samples at different time points. Compared with NT-proBNP, sST2 demonstrated significantly lower coefficients of variation and reference change values. The serum concentration of sST2 is not influenced by sex, age, BMI, renal function, atrial fibrillation, or prior HF diagnosis [65]. Therefore, sST2 may be a good biomarker for monitoring patients with such comorbidities.

However, there are several limitations to the use of sST2 in the clinical setting. First, there are no large, well-designed, prospective studies using sST2. Although many animal experiments have validated sST2, the number of clinical trials is small and most studies have enrolled only a small number of patients. Second, no clear standard value of the sST2 level has been accepted worldwide, with the reference value varying from study to study. In an analysis of the data from the HF-ACTION study, an sST2 cutoff value of 35 ng/dL well predicted short-term all-cause death, cardiovascular death, and HF hospitalization [60]. In the PRIDE study, a multivariable analysis showed that an sST2 concentration above 20 mg/dL strongly predicted 1-year mortality in dyspneic patients [66]. Because the enrolled patients and listed values vary widely from study to study, it is difficult to determine a specific value for use in clinical practice. In addition, the standard values vary widely with sex, being much higher in males than females [67]. Third, sST2 is associated with measures of inflammation, such as leukocyte count, and C-reactive protein, unlike natriuretic peptides [63]. sST2 also exhibits a circadian rhythm and is usually low in the morning and high in the late afternoon, so values may vary depending on the time the blood sample is taken [68].

3. Galectin-3

Galectin-3 is produced by macrophages that stimulates the

Table 4. Clinical trials using sST2

Author	Study population	Aim of study	Implication
Chronic HF			
Ahmad et al. [69]	(HF-ACTION trial) Chronic HF with LVEF below 35% (n=813)	To determine whether biomarkers improve prediction of the mode of death in patients with chronic HF	Predictor of pump failure risk
Gaggin et al. [62]	Chronic HF with LV systolic dysfunction (LVEF <40%) (n=151)	To perform head-to-head comparison of 3 biomarkers (sST2, GDF-15, hs-troponin T)	Only serial measurement of sST2 appeared to add prognostic information to the baseline concentration and predict change in LV function
O'Meara et al. [70]	(PARADIGM-HF trial) HFrEF (LVEF <40%) (n=1,758)	To determine the relationship between sST2 and outcomes and the prognostic utility of various sST2 partition values	Baseline sST2 remained an independent predictor of outcomes. Changes in sST2 from baseline to one month were independently associated with outcome risks
Felker et al. [60]	(HF-ACTION trial) Chronic HF with LVEF below 35% (n=910)	To evaluate ST2 levels and their association with functional capacity and long-term clinical outcomes	ST2 was modestly associated with functional capacity and significantly associated with outcomes
Acute HF			
Manzano-Fernandez et al. [71]	ADHF (n=447)	To determine whether the risk of mortality associated with sST2 concentration differs in ADHF patients with HFpEF compared with patients with systolic HF	sST2 was an independent predictor of mortality, regardless of LVEF
Shah et al. [61]	Acute dyspneic patients with/without decompensated HF (n=139)	To evaluate the associations between sST2 and cardiac structure and function To determine whether sST2 retains prognostic meaning	sST2 was associated with cardiac abnormalities, a more decompensated hemodynamic profile, and long-term mortality
Mueller et al. [72]	ADHF patients in the emergency department (n=137)	To evaluate the value of sST2 as a prognostic marker in patients with ADHF	Increased sST2 levels were independently and strongly associated with 1-year all-cause mortality
Rehman et al. [63]	Patients with acute HF (n=346)	To examine patient-specific characteristics of ST2 in acute HF	As a myocardial-specific response to stretch, ST2 showed strong clinical and biochemical correlations in patients with acute HF. Prognostically, ST2 is powerful in acute HF
Kim et al. [73]	Patients hospitalized with ADHF and renal insufficiency (n=66)	To investigate the role of sST2 as a prognosticator in patients hospitalized with acute HF and renal insufficiency	The pre-discharge sST2 measurement can be helpful in predicting short-term outcomes in ADHF with renal insufficiency

HF, heart failure; LV, left ventricular; LVEF, LV ejection fraction; sST2, soluble suppression of tumorigenicity 2; GDF-15, growth differentiation factor-15; hs-troponin, high-sensitivity troponin; PARADIGM-HF trial, Prospective Comparison of ARNI (Angiotensin Receptor–Neprilysin Inhibitor) with ACEI (Angiotensin-Converting–Enzyme Inhibitor) to Determine Impact on Global Mortality and Morbidity in Heart Failure trial; HFrEF, heart failure reduced ejection fraction; HF-ACTION trial, heart failure: a controlled trial investigating outcomes of exercise training trial; ADHF, acute decompensated heart failure; HFpEF, heart failure with preserved ejection fraction.

profibrotic pathway, leading to proliferation of fibroblast and consequent collagen deposition [74]. In the inflammatory response and wound healing process, pro-inflammatory cytokines released by cardiomyocytes lead to macrophage activation, and activated macrophages release galectin-3, which binds to myofibroblasts, activating them and triggering collagen synthesis. Collagen deposition causes myocardial scarring, long-term remodeling, and dilatation of the LV [75]. Galectin-3 thus promotes the differentiation of fibroblasts into myofibroblasts through both the transforming growth factor-β1-dependent and -independent pathways [76].

Since galectin-3 reflects cardiac function and is a good indicator of HF prognosis, it can be a useful biomarker for HF. Among dyspneic patients with and without acute decompensated HF, elevated galectin-3 are associated with E/Ea ratio, a lower right ventricular function, higher right ventricular systolic pressure, and more severe valvular regurgitation [77]. In a recent study of large population-based cohort, galactin-3, BNP, and sST2, galectin-3 showed the

highest discrimination value for preclinical diastolic dysfunction [78].

Compared with the biological variability of biomarkers between patients with stable HF and healthy adults, galectin-3 showed lower intraindividual biological variability than other biomarkers [74]. In terms of serial measurements, galectin-3 also has advantages over other biomarkers. Serial measurements of biomarkers (NT-proBNP, troponin, sST2, and galectin-3) at different time points in patients with acute HF showed that only galectin-3 was constant over time [79]. Therefore, alterations in galectin-3 level can indicate underlying pathophysiological changes that could lead to a poor prognosis. A previous meta-analysis assessed the usefulness of galectin-3 in predicting short-term all-cause and cardiovascular mortality in patients with HF. The results showed that increased galectin-3 was associated with higher short-term all-cause mortality and cardiovascular mortality, even after adjusting for other well-established risk factors [80]. In a sub-study of the Coordinating Study Evaluating Outcomes of Advising and Counseling in Heart Failure trial, higher galectin-3 identified HF patients at low risk for 1-month and 6-month mortality and HF rehospitalization after an episode of acute HF [81]. In another large-scale cohort analysis (Framingham Offspring Cohort), a higher concentration of galectin-3 was associated with an increased risk of incident HF and short-term mortality [82]. Galectin-3 measurements repeatedly appeared to be a powerful predictor for outcomes in acute HF patients and was independent of NT-proBNP. Galectin-3 might also be useful in clinical practice for prognosis development and treatment monitoring [83]. In HFpEF patients, galectin-3 is also an independent predictor for the outcome of HF and appears to be particularly useful [84].

However, no randomized controlled trials have demonstrated that galectin-3 can be used to accurately diagnose HF or evaluate its prognosis. Although studies differ, galectin-3 seems to have a relatively low diagnostic value in predicting death and HF-readmission [84]. In a community-based cohort, this protein was associated with new-onset HF in the high-risk but not in the low-risk group [85]. Therefore, its usefulness as a biomarker to discriminate HF in outpatients is limited. In addition, no clear standard cutoff value for galectin-3 levels has been accepted worldwide. Galectin-3 levels also correlate with age, BMI, sex, diabetes mellitus, renal dysfunction, and hypertension [86]. In particular, the effect of renal function is significant, and care must be taken in interpretation. According to animal studies, galectin-3 is markedly upregulated in acute tubular injury and the subsequent regeneration [87] as well as in progressive renal fibrosis [88].

Future directions for biomarkers for HF

1. Emerging biomarkers in HF

Given the trend of combining biomarkers, the discovery of novel biomarkers is important. Many HF biomarkers have already been established or are emerging according to multiple pathophysiologic processes [89]. Given the complexity, combining biomarkers that reflect various mechanisms could theoretically offer an improved picture of the myocardial status. Inflammation is thought to play a crucial role in the complex etiology of HFpEF, and the use of biomarkers that reflect inflammation is expected to be key in the future. New biomarkers such as GDF-15, myeloperoxidase, copeptin, and tumor necrosis factor lack clinical validation, but are soon expected to play a role as HF biomarkers.

2. Multiple biomarker approach

Regardless of the type of biomarker, combining biomarkers can improve the accuracy of diagnosis, prognostication, and assessment of treatment effects. Most biomarkers maximize their usefulness when used in combination with NT-proBNP levels. In particular, the combination of ST2 and NT-proBNP has been shown to be an excellent predictor of prognosis [63]. Troponin T also showed good results when measured together with NT-proBNP. The risk of HF is significantly greater when there is an increase in both biomarkers compared with an increase of either NT-proBNP or troponin T alone [90]. Even without BNP, combinations of biomarkers have a considerable value. In chronic stable HF, elevated sST2 and galectin-3 had a significantly higher hazard ratio together than alone, regardless of the BNP level, suggesting that simultaneous sST2 and galectin-3 elevation is associated with poor prognosis [91]. In addition, hs-C-reactive protein, which represents inflammation, can be used as a parameter for HF, and research on this has been reported recently [92]. Strategies that combine multiple biomarkers may ultimately give benefits in guiding HF therapy, but additional validation is needed [93,94].

3. Serial measurements of biomarkers

Although biomarkers can differ between patients, it is important to check whether a particular patient's levels increase compared with the baseline level. Serial measurements of biomarkers are important for the diagnosis of HF in the community. According to an observational study (Cardiovascular Health Study), the frequency of biomarker increase per year in the HF-free population is 14.8% for troponin T and 13.2% for NT-proBNP. After 10 years, the cumulative HF incidence is 50.4% when there is an increase in both biomarkers, and 12.2% when neither biomarker is increased [90]. Therefore, even if at a particular time HF was not diagnosed because of normal biomarker levels, a diagnosis becomes more likely in follow-up biomarker measurements.

Serial biomarker follow-up is important for predicting HF outcomes. It was found that serial measurements of natriuretic peptides provide strong prognostic information in chronic HF, not only in HFrEF but also in HF with mid-range EF and HFpEF [95-97]. A previous study analyzing two independent randomized controlled trials of chronic HF (Val-HeFT and GISSI-HF trials, a total of 5,284 patients) reported that changes in hs-troponin T concentration over time were a robust predictor of future cardiovascular events in patients with chronic HF [98]. In addition, changes in biomarkers over time in chronic HF can predict the risk of adverse events or outcomes, as well as changes in cardiac structure or LV function [99]. Changes in the concentration of biomarkers are likely to reflect the presence of ongoing cardiac pathophysiology, and could offer a mechanism to differentiate preclinical HF phenotypes.

4. For healthcare providers

To increase the efficiency of biomarker measurement, physicians must select appropriate biomarkers that represent each patient's clinical characteristics. In addition, the type of biomarker and cutoff value implemented by each center may be different. In particular, in the case of natriuretic peptides, BNP, proBNP, and NT-proBNP have different clinical implications, so care must be taken not to confuse them. Biomarkers that reflect myocardial injury will be helpful to evaluate the degree of myocardial damage caused by acute events. After HF progresses, biomarkers that reflect cardiac remodeling, such as hypertrophy or fibrosis, might provide the most information. However, discovering clinically appropriate biomarkers is difficult because of the many mechanisms involved in the various HF phenotypes. Physicians should consider the confounding aspects of biomarkers and interpret their values with caution. All biomarkers can be significantly affected by various factors, and the severity of HF, general condition, and comorbidities must be carefully considered. Particularly, in cases of acute HF with hemodynamic instability, the timing of laboratory tests can greatly affect the results. Within a single patient, biomarker levels can vary with time, depending on the use of diuretics, hemodialysis, mechanical ventilation, etc. The multi-marker approach to HF management and decision-making is useful in the emergency room and offers various cutoff values for each biomarker [3].

5. Future directions in clinical trials

Several biomarkers are recommended in the current guidelines. However, while BNP and NT-proBNP are employed, the clinical use of other biomarkers is still limited owing to a lack of adequate clinical trials. In addition, the clinical setting of the enrolled patients varies among the few available studies, and the number of enrolled patients is too small. The reference value can also vary depending on the equipment used to analyze the samples, so inter-equipment validation is required. Therefore, further large-scale biomarker studies are warranted.

Currently, in Korea, a large-scale registry (KorHF III Registry, under the leadership of the Korea HF Society) that includes various biomarkers (BNP, NT-proBNP, ST2, cardiac troponin I, and cardiac troponin T) is in progress and is likely to provide important information.

However, the combination of biomarkers from different pathophysiological processes remains unknown. Moreover, we do not know when and how often these biomarkers should be measured for appropriate management of patients with HF. An increased number of biomarker tests correlates with a better HF diagnosis, but physicians must consider cost-effectiveness in clinical practice. Thus, it is difficult to conduct multiple biomarker tests that are not included in the guidelines. We should study which biomarkers to combine, when to measure, and how often to measure. In addition, we should consider their cost-effectiveness, balancing the cost of testing and their benefit in appropriate HF management.

Conclusions

Despite several limitations, BNP/NT-proBNP are the only biomarkers included in the current guidelines. Cardiac troponins, sST2, and galectin 3 are independent prognostic biomarkers of HF and can be used as supplementary measurements. Although clinical trials are needed to reasonably apply these biomarkers in clinical practice, it is essential to add new biomarkers to the guidelines to assist and support healthcare providers in managing HF.

Future research should adopt a multi-marker approach to improve the risk prediction models, diagnosis, and management of HF. When using biomarkers for HF, it is also important to establish a setting for multiple tests to evaluate risk stratification or predict prognosis, rather than relying on a single test. To better use cardiac biomarkers, physicians must select appropriate biomarkers for HF and exert caution when interpreting their values considering variable clinical profiles.

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Adverse events following vaccination against coronavirus disease 2019

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To overcome the coronavirus disease 2019 (COVID-19) pandemic, large-scale vaccination is proceeding worldwide. As of December 23, 2021, 10 novel vaccines against COVID-19 had been validated for use by the World Health Organization (WHO), including BNT162b2 (Pfizer/BioNTech), mRNA-1273 (Moderna), AZD1222 (AstraZeneca), and Ad26.COV2.S (Janssen). These novel vaccines against COVID-19 showed acceptable safety profiles in randomized clinical trials. Most adverse events following immunization (AEFIs) associated with these novel vaccines ranged from mild to moderate and improved within a few days after administration. However, serious adverse events associated with vaccines that were not observed in the clinical trials were reported in real-world data. Adverse events of special interest include not only anaphylaxis or neurologic disorders (such as Guillain-Barré syndrome, transverse myelitis, or seizure) but also myocarditis or pericarditis associated with the messenger RNA (mRNA) vaccines and thrombosis with thrombocytopenia syndrome associated with the adenovirus-vector vaccines. Although several fatal cases of serious AEFIs that may have been related to vaccination have been reported, it is recommended to continue vaccination because the benefits of vaccines' preventive effects against COVID-19 outweigh the risks of rare serious adverse events. Long-term monitoring of various AEFIs and sharing of clinical experiences are necessary for safe and efficient large-scale vaccination.

Keywords: Adverse drug events; COVID-19; Vaccination

Introduction

On December 31, 2019, the first case of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2, was reported in Wuhan City, China. Since then, the number of COVID-19 cases has continuously increased, reaching a total of 373,229,380 confirmed cases, including 5,658,702 deaths, reported to the World Health Organization (WHO) as of January 31, 2022 [1]. To overcome the pandemic, large-scale vaccination is implemented at an unprecedented rate worldwide. Until December 23, 2021, several novel vaccines against COVID-19 were validated for use by the WHO (Emergency Use Listing), as follows: BNT162b2 (Pfizer/BioNTech), AZD1222 (AstraZeneca), Ad26.COV2.S (Janssen), mRNA-1273 (Moderna), BBIBP-CorV (Sinopharm), CoronaVac (Sinovac), BBV152 (Bharat Biotech), and NVX-CoV2373 (Novavax) [2]. The three vaccines were approved for the prevention of COVID-19 by the

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U.S. Food and Drug Administration (FDA): BNT162b1 (Pfizer/BioNTech), mRNA-1273 (Moderna), and Ad26.COV2.S (Janssen) [3].

The adverse event following immunization (AEFI) is defined as any unexpected medical situation which follows immunization. It does not definitely have a causal relationship with the usage of the vaccine. It can either be truly resulting from vaccine or immunization process or coincidentally occurred caused by something other than the vaccine products, immunization error or anxiety [4]. Although randomized controlled trials have demonstrated the safety of these vaccines [5-8], several cases of severe AEFIs have been reported. To manage various AEFIs and assess the safety of novel vaccines, the government in each country monitors and discloses information about adverse events associated with vaccination [9-11]. Following the reported serious AEFIs associated with novel vaccines, strategies and policies of national vaccination have been changed; however, there are also people that still refuse to be vaccinated, concerned about serious adverse events. In this review, we consider the types and frequencies of AEFIs of four COVID-19 vaccines in use in the Republic of Korea: BNT162b2, mRNA-1273, AZD1222, and Ad26.COV.2.S.

BNT162b2 (Pfizer/BioNTech)

A total of 43,548 participants aged 16 years and over were enrolled in a randomized controlled trial to evaluate the safety and efficacy of BNT162b2 [7]. In the vaccinated group, mild-to-moderate pain at the injection site was the most commonly reported local AEFI, which was reported less frequently by older participants (>55 years of age) than by younger participants and more often after the first dose than the second. The most commonly reported systemic AEFIs were fatigue and headache after either dose in all the age groups. Systemic AEFIs, including fever, fatigue, headache, muscle pain, and joint pain, were reported more often by younger participants than by older participants and often after the second dose. In general, mild-to-moderate local and systemic AEFIs were observed within the first 1–2 days after vaccination and resolved shortly thereafter. In the vaccinated group, shoulder injuries related to vaccine administration, axillary lymphadenopathy, paroxysmal ventricular arrhythmia, and leg paresthesia were reported. Two vaccine recipients died during the study period, and no deaths were considered related to the vaccine [7,12]. In an ongoing trial with 6 months of follow-up after vaccination, BNT162b2 have had an acceptable safety profile. Nevertheless, new AEFIs attributable to the vaccine include decreased appetite, lethargy, asthenia, night sweats, and hyperhidrosis; no cases of myocarditis were noted in this trial [13].

Between December 14 and 23, 2020, the Centers for Disease Control and Prevention (CDC) and the FDA confirmed 21 cases of anaphylaxis reported through the Vaccine Adverse Event Reporting System after administration of 1,893,360 doses of BNT162b2 in the United States. The incidence of anaphylaxis was estimated to be 11.1 events per million doses. Most cases occurred within 30 minutes after administration. Four patients (19%) were hospitalized, and no deaths were reported. Seven out of 17 patients (41%) with a history of allergy had experienced anaphylactic reactions in the past, including reactions to vaccines. Polyethylene glycol (PEG), a component of BNT162b2 and mRNA-1273, has the potential to elicit allergic reactions [14]. PEG is frequently used as an excipient in various products, including bowel preparation medication, cosmetics, toothpaste, and contact lenses. Recently, a series of confirmed PEG allergies has been reported. PEG has then been described as an allergen hidden in drugs, which might be underdiagnosed [15]. Therefore, people who have experienced anaphylaxis associated with PEG are contraindicated in BNT162b2 vaccination.

It has been suggested that myocarditis and pericarditis may occur as cardiovascular adverse events associated with messenger RNA (mRNA) COVID-19 vaccines. Using national data from health care organizations in Israel, Barda et al. [16] reported that BNT162b2 vaccination was associated with an elevated risk of myocarditis (risk ratio, 3.24; 95% confidence interval [CI], 1.55–12.44); however, the risk of myocarditis was substantially increased after COVID-19 (risk ratio, 18.28; 95% CI, 3.95–25.12). In this study, the incidence of myocarditis was analyzed to be 2.2 events after vaccination and 10.3 events after COVID-19 per 100,000 persons (21/398,812 of vaccinated group vs. 19/183,710 of COVID-19 group). According to a systematic review of cardiac complications following mRNA COVID-19 vaccines, myocarditis and pericarditis were the most common adverse events among the 243 reported cardiac complications following mRNA COVID-19 vaccine administration up to September 25, 2021. Among them, 227 were diagnosed with

myocarditis. After receiving BNT162b2, 74.4% of myocarditis cases were reported after the second dose. The median age was 21 years (range, 12–70 years), and 92.1% of the patients were male. The most commonly reported symptoms were chest pain (96.1%), fever (38.2%), myalgia (18.8%), and shortness of breath (10.1%). Seven patients were diagnosed with pericarditis after BNT162b2 vaccination. The median age was 37 years (range, 21–80 years), and 71.4% of the patients were male. The most commonly reported symptom was chest pain (85.7%). Cardiac biomarkers (creatine kinase-myocardial band, troponin, and N-terminal prohormone of brain natriuretic peptide) and inflammatory markers (C-reactive protein and erythrocyte sedimentation rate) were elevated in most patients with myocarditis. However, all patients with pericarditis showed a normal range of troponin levels and elevated inflammatory markers. Cardiac magnetic resonance imaging the gold standard approach for diagnosing myocarditis, was abnormal in all patients diagnosed with myocarditis. Regarding transthoracic echocardiography, reduced left ventricular ejection fraction and pericardial effusion were commonly reported in myocarditis and pericarditis cases, respectively. Nonsteroidal anti-inflammatory drugs, colchicine, and steroids were commonly prescribed to manage both complications [17].

In Korea, according to the weekly report of adverse reaction after COVID-19 vaccination, 51st weeks published by Korea Disease Control and Prevention Agency reported by February 24, 2022, 319.8 adverse events and 12.7 serious adverse events were reported per 100,000 BNT162b2 vaccinations. Serious adverse events included death, anaphylaxis, admission of intensive care unit, critical ill condition, permanent disability and adverse event special interest. By February 20, 2022, 72,217,274 doses were administered. Five hundred and seventy-one cases of anaphylaxis were reported and 79.1% occurred after first dose of vaccination. Three hundred and sixty-one cases of myocarditis/pericarditis were reported. Among them, 61.5% were male and 70.9% were under the age of 40 which were similar to the reports from other countries [18].

mRNA-1273 (Moderna/NIAID)

According to a phase III randomized, observer-blinded, placebo-controlled trial conducted with 30,420 persons aged 18 years or older to evaluate the safety and efficacy of mRNA-1273, solicited local and systemic AEFIs was reported more often in the vaccinated group after both the first and second doses than in the placebo group [5]. In the vaccinated group, injection site pain was frequently reported after the second dose. Delayed injection site reactions with an onset from day 8 onwards, including erythema, induration, and tenderness, were reported to be 0.8% and 0.2% after the first and second doses, respectively, which resolved over the following 4–5 days. Systemic AEFIs, such as fatigue, myalgia, and headache, were more severe and frequent after the second dose than the first dose. Both local and systemic AEFIs were more common among the younger group aged under 65 years than the older group aged 65 years and over. The frequency of the serious adverse events was similar between the vaccinated and placebo groups. Hypersensitivity events and Bell's palsy were reported in 1.5% and <0.1% of the vaccinated groups, respectively, which was similar to the placebo group. Of the vaccinated participants, there were three cases of cerebrovascular accident (one in placebo), two of embolic stroke (none in placebo), one of transient ischemic attack (none in placebo), and two of deep vein thrombosis (none in placebo). However, none of these events were considered AEFIs by the investigator, as it was evident that all subjects had a significant medical history or increased risk for these events. No evidence of vaccine-associated death was reported [5,19].

According to the CDC's report of allergic reactions after administration of mRNA-1273, between December 21, 2020, and January 10, 2021, 4,041,396 doses of the vaccine were administered. Ten cases were diagnosed as anaphylactic reactions in the United States (2.5 per million of mRNA-1273 vaccines administered). Among them, six patients required hospitalization, and no deaths were reported [20].

According to a systematic review of cardiac complications following mRNA COVID-19 vaccines, the frequency of myocarditis was lower after administration of mRNA-1273 than after administration of BNT162b2 (25.6% and 74.4%, respectively) [17]. Patone et al. [21] reviewed COVID-19-vaccinated people aged 16 years or older between December 1, 2020, and August 24, 2021, in England to investigate the risk of cardiac AEFIs associated with COVID-19 vaccines on 1–28 days following vaccination (AZD1222, n=20,615,911; BNT162b2, n=16,993,389; mRNA-1273, n=1,006,191). The authors reported an extra 6 and 10 myocarditis events per 1 million people vaccinated with the first and second doses

of mRNA-1273, respectively. This number is much lower than the 40 myocarditis events per 1 million patients (95% CI, 38.0–41.0) following COVID-19. In the subgroup analysis, the risk of myocarditis following mRNA vaccination was the highest in those aged <40 years.

In phase III clinical trial, facial swelling was reported in two participants in the vaccinated group (one in placebo) and they received dermal filler injection prior to vaccination, which resolved within a week [19]. Rare cases of cutaneous reactions, such as erythematous and tender swelling, associated with hyaluronic acid dermal fillers and mRNA-1273 have been reported. These cases resolved spontaneously or were successfully managed with oral steroids. These may represent a delayed hypersensitivity reaction to an immunological trigger following vaccination [22-24].

In Korea, until February 20, 2022, 23,515,972 doses were administered. Adverse events and serious adverse events were reported at 461.4 and 9.9 per 100,000 mRNA-1273 vaccinations, respectively. One hundred and twenty-one cases of anaphylaxis were diagnosed; among them, 80.2% occurred after the first dose of vaccination and 51.2% were female. None of thromboembolic adverse event was reported after mRNA-1273 vaccination. One hundred and seventy-one cases of myocarditis/pericarditis were reported. Among them, 60.8% were male and 67.8% were under the age of 40. Compared with BNT162b2, the incidence of myocarditis/pericarditis in the under the age of 19 group was lower after mRNA-1273 vaccination (4.1% [7/171] vs. 28.3% [102/361], respectively) [18]. Currently, the ages at which BNT162b2 and mRNA-1273 can be administered are over 12 years old and over 18 years old, respectively [25]. Therefore, it is possible that the incidence of myocarditis/pericarditis after BNT162b2 vaccination was higher than after mRNA-1273 vaccination because administration rate of BNT162b2 was higher than mRNA-1273 in the age group under 19 years of age. No data could be found that the report of facial swelling related to mRNA-1273 vaccination after receiving dermal filler injection until February 2022, but continuous monitoring is needed.

AZD1222 (AstraZeneca/Oxford)

To evaluate the safety and efficacy of AZD1222, four ongoing blinded, randomized controlled trials were analyzed; COV001-phase I/II, COV002 phase II/III, COV003-phase III, and COV005-phase I/II [8,26-29]. Among the 12,021 recipients of AZD1222, the most commonly reported AEFIs were injection-site tenderness, headache, and fatigue. The AEFIs after the second dose were mild and less frequent than those after the first dose. AEFIs were reported less frequently in participants aged 65 years and older than in those aged 18–65 years. The frequency of serious AEFIs was 0.7% in the vaccinated group compared with 0.8% in the placebo group. Out of the reported 175 AEFIs, a case of transverse myelitis and fever higher than 40°C was considered to be related to AZD1222. Interestingly, adverse events of special interest (AESI) were reported less frequently in the vaccinated group than in the placebo group (0.8% and 1.1%, respectively). The definition of AESI is that a preidentified and predefined medically-significant event that has the potential to be causally associated with a vaccine product that needs to be carefully monitored and confirmed by further specific studies. AESI defined for COVID-19 vaccines include the following: vaccine-associated enhanced disease, multisystem inflammatory syndrome in children, acute respiratory distress syndrome, acute cardiovascular injury, coagulation disorder, acute kidney injury, generalized convulsion, Guillain-Barré syndrome (GBS), acute liver injury, anosmia/ageusia, chilblain-like lesions, single organ cutaneous vasculitis, erythema multiforme, anaphylaxis, acute aseptic arthritis, meningoencephalitis, acute disseminated encephalomyelitis, and thrombocytopenia [30]. In the vaccinated group, anaphylaxis (<0.1%), neurological events (0.5%), and thrombotic/thromboembolic events (<0.1%) were reported as AESI [8,26,27].

Although no related cases of anaphylaxis were reported in clinical trials, they also occurred. In Korea, as of January 23, 2022, 20,499,046 doses of AZD1222 had been administered, and 325 cases (1.6%) were reported suggesting anaphylactic reaction; 285 (87.7%) after the first dose and 39 (12.0%) after the second dose [10]. Polysorbate 80, an excipient of AZD1222, has been suggested as the cause of hypersensitivity reaction. Polysorbate is an excipient in hepatitis B, influenza, and human papillomavirus vaccines. PEG and polysorbate have the same structure and can represent cross-reactivity [31].

Thrombosis with thrombocytopenia syndrome (TTS) is a rare but serious adverse event associated with AZD1222. As of March 20, 2021, five patients presented with severe venous thromboembolism at unusual sites with thrombo-

cytopenia within 10 days after receiving the first AZD1222 dose. All these patients showed high levels of antibodies to platelet factor (PF)4-polyanion complexes [32]. As of April 7, 2021, 11 cases of thrombosis with thrombocytopenia following the first dose administration of AZD1222 were reported in Germany and Austria. Thrombotic events included cerebral venous thrombosis, splanchnic vein thrombosis, and pulmonary embolism, and six patients died. The serum obtained nine of 11 patients showed strong reactivity on the PF4-heparin enzyme-linked immunosorbent assay [33]. Initially, these cases were referred as vaccine-induced immune thrombotic thrombocytopenia, as their clinical features are similar with heparin-induced thrombocytopenia [32]. TTS was characterized by mainly occurring as cerebral venous sinus thrombosis or splanchnic vein thrombosis after 4 days to 2 weeks from AZD1222 administration, concomitant with thrombocytopenia without recent exposure of heparin. It was hypothesized that vaccine-induced antibodies lead to thrombosis through platelet activation [34]. The European Medicines Agency (EMA) registered TTS as a very rare adverse event of AZD1222 in the product description. However, the EMA and the Medicines and Healthcare products Regulatory Agency confirmed that the benefits of AZD1222 against COVID-19 continue to outweigh the risk of adverse events; therefore, continuous vaccination is recommended [35,36]. According to the Yellow Card Reports in the United Kingdom up to January 25, 2022 (24.9 million first doses, 24.2 million second doses), 436 cases of major thromboembolic events with thrombocytopenia following vaccination with AZD1222 were reported (88.8% after the first dose and 11.2% after the second dose). The overall case fatality rate was 18% [11].

GBS is a rare neurological disorder associated with infection or immunization. As of July 31, 2021, 833 cases of GBS were reported following AZD1222 vaccination, while approximately 592 million doses of AZD1222 were administered worldwide by July 25, 2021. Based on the assessment of these data, the EMA determined that a causal relationship between AZD1222 and GBS is at least a reasonable possibility. Therefore, GBS was added to the product information as a rare side effect of AZD1222 [37]. After AZD1222 vaccination, few cases of encephalitis or transverse myelitis have been reported, and they are still extremely rare (<1 report per 100,000 doses) [38,39]. In the case of serious neurological adverse events are reported after the first dose of AZD1222, administration of the second dose is not recommended.

Capillary leak syndrome (CLS) is a rare disorder associated with the recurrent episodes of extravasation of fluid and protein into the interstitial space from capillaries, resulting in swelling of arms and legs. The triad of hypotension, hemoconcentration, and hypoalbuminemia supports the diagnosis of CLS. Nevertheless, the exact pathophysiology of CLS remains unclear [40]. The EMA disclosed an estimated reporting rate of one case of CLS for more than 5 million doses of AZD1222. Although the causality between AZD1222 and CLS has not been fully demonstrated, the EMA concluded that CLS should be added to the product information of AZD1222 as an adverse event with unknown frequency. AZD1222 is contraindicated in patients who have previously experienced of CLS [37].

In Korea, until February 20, 2022, 20,348,850 doses were administered. Adverse events and serious adverse events were reported at 536.8 and 27.6 per 100,000 AZD1222 vaccinations, respectively. One hundred and eight cases of anaphylaxis were diagnosed; among them 95.0% occurred after the first dose of vaccination. Three cases of TTS were diagnosed, all occurring after the first dose of AZD1222 vaccination. One female in her seventies was diagnosed with deep vein thrombosis, two males in their thirties were diagnosed with cerebral vein thrombosis (CVT) and one died [18]. As a result, to reflect the risk of this fatal adverse event, the AZD1222 vaccination policy was revised to be recommended for those aged 50 and over as of July 2021 [41]. By February 18, 2022, 21 suspicious cases of GBS associated with COVID-19 vaccination were reviewed and 19 cases were reported after AZD1222 vaccination (first dose, 15 cases; second dose, 4 cases). In these 19 cases, there was a time probable relationship with vaccination and onset of adverse events, but it was determined that there were insufficient data of causal relationship between vaccine and adverse event. No case of CLS after AZD1222 vaccination was reported [18].

Ad26.COV2.S (Janssen)

A total of 39,321 participants who were subdivided into two age groups (18–59 years of age group and ≥60 years of age group) were enrolled in a randomized, double-blind, placebo-controlled, phase III trial to evaluate the safety and

efficacy of a single dose of Ad26.COV2.S vaccine [6]. Among them, 6,736 participants were included in the safety analysis during the 7 days after the administration of Ad26.COV2.S or placebo. The most commonly reported local and systemic AEFIs in the vaccinated group were injection-site pain and headache, respectively. More AEFIs were reported in the 18–59 years age group than in the ≥60 years of age group. The frequency of serious AEFIs considered related to the Ad26.COV2.S vaccine was similar between the vaccinated group and the placebo group (0.4% and 0.4%, respectively). In the vaccinated group, one patient had GBS, one pericarditis, two Bell's palsy, and one brachial radiculitis were reported. Notably, 11 cases of venous thromboembolism (three in placebo), four of seizure (one in placebo), and six of tinnitus (none in placebo) were reported in the vaccinated group. However, the authors could not determine a causal relationship between these events and Ad26.COV2.S. There were three deaths in the vaccinated group (16 in the placebo group), considered unrelated to the trial intervention [6]. In the final analysis of single-dose Ad26.COV2.S, one case of TTS was reported in the vaccinated group. No cases of CLS, myocarditis, or encephalitis have been reported [42].

From March 2 to April 21, 2021, in the United States, 12 cases of cerebral venous sinus thrombosis were reported among recipients of the Ad26.COV2.S vaccine. Similar to those of another adenovirus-vector vaccine, AZD1222, these events occurred primarily in females within 2 weeks of administration and were accompanied by thrombocytopenia. Eleven patients were confirmed positive for PF4-antibody, and three patients died [43]. As a result, vaccination of Ad26.COV2.S was temporarily paused in the United States, but it was resumed because the benefit of preventing COVID-19 was higher than the risk of a very rare adverse event [44]. Following the reports of updates on TTS following Ad26.COV2.S vaccination from March 2 to December 9, 2021 by CDC, 54 cases were reported and eight patients died. Most cases were women and under 50 years of age. The median time from vaccination to symptom onset was 9 days (range, 0–18 days). The CDC disclosed that the reporting rate of TTS following Ad26.COV2.S vaccination was 3.8 per million doses, and the proportion of TTS cases with death did not decrease after the vaccination pause on April 13, 2021 [45]. According to the results of an observational study in Europe using the EudraVigilance database, between January 1 and July 30, 2021, 761 cases of thrombotic events were reported (10,972,234 doses of Ad26.COV2.S administered). The reporting rate of CVT per 1 million person vaccinated-days was 11.48 (95% CI, 9.57 to 13.67) for Ad26.COV2.S., 21.60 (95% CI, 20.16–23.11) for AZD1222, 1.92 (95% CI, 1.71–2.12) for BNT162b2, and 5.63 (95% CI, 4.74–6.64) for mRNA-1273. Similar to the results of a report in the United States, the highest prevalence of CVT was observed in the under 65 years and female groups [46].

Transverse myelitis is an inflammation of parts of the spinal cord. By August 31, 2021, 11 cases of transverse myelitis were considered to have at least a possible or probable causal relationship with the Ad26.COV2.S vaccination (more than 33 million doses of the Ad26.COV2.S vaccine had been administered worldwide). Therefore, the EMA recommended the addition of inflammation of the spinal cord as a side effect with unknown frequency to the product information. GBS was also recommended to be added as a very rare side effect (it may affect up to 1 in 10,000 people) [47,48].

Similar to other adenovirus-vector vaccines (AZD1222), Ad26.COV2.S vaccines are not recommended for people who have previously had CLS. The EMA reviewed three cases of CLS following Ad26.COV2.S vaccination, and two of them died. Therefore, the Committee recommended that this vaccine should not be administered to people with a history of CLS, and healthcare professionals should be aware of the risk of CLS following vaccination, which can be fatal if untreated [49].

In Korea, until February 20, 2022, 1,508,231 doses were administered. Adverse events and serious adverse events were reported at 585.6 and 26.4 per 100,000 Ad26.COV2.S vaccinations, respectively. Thirty cases of anaphylaxis occurred and 66.7% were male; it may because the Ad26.COV2.S was primarily administered to Korean soldiers. No cases were reported with TTS or transverse myelitis. Two cases of GBS were reported after Ad26.COV2.S vaccination, which were determined that there were insufficient data of causal relationship between vaccine and GBS [18]. There was one case of fatal CLS after Ad26.COV2.S administration in patient with multiple myeloma [50]. This case was reviewed and finally demonstrated probably not related with vaccination because of the lack of data [18].

Conclusions

According to recent reports, the novel vaccines against

COVID-19 have an acceptable safety profile. Continuous vaccination is recommended, as the benefits outweigh the risks of rare serious adverse events. Long-term monitoring of various AEFIs and sharing clinical experience are necessary for safe and efficient large-scale vaccination.

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Sleep and vaccine administration time as factors influencing vaccine immunogenicity

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The immunogenicity of vaccines is affected by host, external, environmental, and vaccine factors; in addition, sleep or circadian rhythms may also have effects. With the use of vaccines to mitigate the coronavirus disease 2019 (COVID-19) pandemic, research is underway to clarify what time of the day is optimal for COVID-19 vaccination and how disturbances of circadian rhythms will affect the immunogenicity of the vaccine in shift workers. Studies on the relationship between sleep time and the immunogenicity of vaccines for influenza and hepatitis have demonstrated that less sleep time and sleep deprivation tended to adversely affect immunogenicity. In some studies, there were even sex differences in these effects. When comparing shift workers with disturbances in their circadian rhythms and those who only worked during the day, one study found less antibody formation in shift workers; however, further studies on the relationship between shift work and the immunogenicity of vaccines are needed. Studies on the relationship between vaccine administration time and immunogenicity have shown different results according to age and sex. Therefore, future studies on vaccine administration time and immunogenicity may require an individualized approach for each vaccine and each population to be vaccinated. There is accumulating evidence on the effects of sleep and vaccine administration time on the immunogenicity of vaccines. However, further studies are needed to determine whether the association between immunogenicity and circadian rhythms and vaccine administration time can be used as a basis to increase the immunogenicity for individual vaccines.

Keywords: Circadian rhythms; Immunogenicity, vaccines; Sleep

Introduction

Vaccination has had a tremendous impact on people's health worldwide. It was possible to prevent countless people from contracting diseases or dying through vaccination [1].

Efforts have been made to create an effective and safe vaccine against infectious diseases that threaten people's health. As people worldwide are being vaccinated owing to the coronavirus disease 2019 (COVID-19) pandemic, information on the immunogenicity and safety of the vaccines is of great interest. Several factors influence the immunogenicity of vaccines. For example, there are intrinsic host factors, such as sex, age, immune history, and comorbidities; environmental factors, such as geographic location and season; nutritional factors, such as body mass index; and vaccine factors, such as the type of vaccine antigen, injection route, adjuvant type, and dose [2].

In addition, sleep or circadian rhythms have been indicated to also affect the immunogenicity of vaccines [3,4].

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The circadian rhythm regulates the sleep-wake cycle of the human body, blood pressure, body temperature, daily changes in cortisol production, and immune function [5,6]. Simultaneously, sleep also affects the immune function [5]. Therefore, researchers have studied whether administering vaccines at a specific time of the day will have a positive effect on the immunogenicity of the vaccines and whether sleep affects such immunogenicity [5,7]. Recently, research results on when it is best to administer COVID-19 vaccines have been reported, and research on the immunogenicity of these vaccines in shift workers is ongoing [8,9].

This review summarizes the results of studies on the effect of sleep or circadian rhythms on the immunogenicity of vaccines. Based on the study findings, we would like to introduce ongoing studies on the relationship between circadian rhythms and immunogenicity of COVID-19 vaccines.

Immune response, circadian rhythm, and sleep

Most vaccines induce B- and T-cell responses, producing antibodies (humoral immunity) and inducing cellular immunity. Antibodies can specifically bind to toxins or pathogens and directly or indirectly kill infected cells with cytotoxic CD8+ T lymphocytes. CD4+ T lymphocytes reduce, regulate, and clear extracellular and intracellular pathogens by assisting B cells, CD8+ T lymphocytes, and macrophages [10].

The first report by Elmadjian and Pincus [11] in 1946 showed that the number of circulating lymphocytes changed throughout the day. Subsequent studies have shown that circadian rhythms affect the regulation of proinflammatory cytokine secretion. For example, when endotoxins stimulate macrophages, the amount of tumor necrosis factor alpha (TNF-α) secreted by macrophages shows a circadian pattern according to the injection time of the endotoxin [12]. However, if there is light-dark cycle desynchronization or a defect in a key gene related to the circadian clock, circadian changes in cytokine production cannot be seen in macrophages. Because clock-controlled genes generate circadian patterns for gene expression in cells, the regulation of metabolism and other major pathways is influenced by the time of day [13,14]. Circadian rhythms also regulate leukocyte trafficking. Rhythmic trafficking in both neutrophils and lymphocytes is driven by clocks regulated by leukocytes and endothelial cells surrounding blood vessels [15,16].

Sleep strengthens innate and adaptive immune responses [17]. Studies have shown that sufficient regular sleep and sleep quality enhance immune function and improve response to vaccines [5]. The secretion of proinflammatory cytokines is increased in cases of experimental sleep deprivation and in people who have a habit of sleeping less, especially men [18,19]. It has been reported that the levels of neutrophils, B lymphocytes, monocytes, and natural killer (NK) cells increase when awake for a long time and decrease after getting enough sleep [7].

Although circadian rhythms and sleep are different domains, they influence each other. Because of this interaction, the lack of sleep in people with circadian rhythm disturbances may affect the immune function [20].

Animal studies on the influence of circadian rhythms and sleep on the immunogenicity of vaccines

Adequate sleep is important for the maintenance of good health in animals. Long-term sleep deprivation in rats has fatal consequences on vitality, and chronic sleep deprivation in animals has led to splenic atrophy and bacteremia [21,22]. There have been several animal studies on the effect of sleep time and vaccine administration time on the efficacy of vaccines. In mice, the response of T lymphocytes to antigens has been reported to exhibit circadian rhythms [23]. The proliferation of T lymphocytes in response to stimulation showed circadian changes, and the tyrosine kinase ZAP70, which plays a key role in T lymphocyte function, also showed rhythmic protein expression [23]. In addition, mice immunized with OVA peptide-loaded dendritic cells (DCs) demonstrated a stronger specific T cell response when immunized during the day than when vaccinated at night. A recent study also showed that when mice were administered DCs loaded with the OVA257–264 peptide antigen (DC-OVA), the CD8+ T cell response was higher when administered in the middle of the day than at other time points [24]. In this study, when the circadian transcriptome of CD8+ T cells was analyzed, it was confirmed that the genes and pathways involved in T cell activation were abundant during daytime.

The activation of innate immunity is essential for in-

ducing an effective adaptive immune response. Silver et al. [25] evaluated the response to a vaccine when CpG oligodeoxynucleotide (ODN)-adjuvanted immunization was performed after Toll-like receptor (TLR) 9 expression and function increased. Antigen-induced proliferation and interferon-gamma production were significantly higher when OVA-CpG ODNs were administered to zeitgeber time (ZT) 19 (nighttime) mice, which had an increased TLR9 response, than when they were administered to ZT7 mice.

Mice are nocturnal animals; therefore, they are active at night and sleep throughout the day. Based on the results of studies on vaccine administration time and immunogenicity in mice, the response of T cells to the vaccine antigen was higher during the day than at night. However, in an experiment on the expression and function of TLR9, a factor related to innate immunity, the immunogenicity was better when mice were vaccinated using TLR9 ligands as an adjuvant than when they were vaccinated at night.

Human studies on the association between sleep and the immunogenicity of vaccines

In human vaccine clinical trials, it has been reported that sleep and shift work patterns affect the immunogenicity of vaccines. Studies on the relationship between sleep duration or sleep deprivation and post-vaccination immunogenicity have mainly been conducted using influenza and hepatitis vaccines. Table 1 summarizes human studies on the relationship between sleep and the immunogenicity of vaccines. Among studies on sleep deprivation and the immunogenicity of vaccines in humans, the earliest results were published in 2002 by Spiegel et al. [26]. In 25 young men with an average age of 23 years, one group of 11 participants slept only 4 hours during the first 6 days, and the other group of 14 participants slept 7.5–8.5 hours as usual. Influenza vaccine was administered on the morning of the 4th day. In the antibody titer measurement 10 days after vaccination, in which the antibody increased logarithmically, the average antibody titer of the sleep-deprived group was less than half of that of the normal sleep group. However, there was no significant difference in the antibody titers between the two groups 3–4 weeks after vaccination. Prather et al. [27] administered influenza vaccine to 83 young adults and asked them to maintain a sleep diary for 13 days before and after vaccination. In their study, the antibody titers to the A/New Caledonia viral strain tested 1 and 4 months after vaccination were lower in those who had a shorter sleep period than in those with a longer sleep period. Lange et al. [28] administered hepatitis A vaccine to 19 adults aged 20–35 years and evaluated the antibody titer 28 days after immunization. Ten participants slept at nighttime as usual on the vaccination day, while nine participants were not allowed to sleep and kept waking for 36 hours on the vaccination day. The hepatitis A vaccine antibody titers were almost twice as high in the participants who slept as usual after vaccination compared with those in the participants who had to remain awake on the day of vaccination. According to the study published in 2011 by Lange et al. [29], in 27 healthy men who were administered hepatitis A and B vaccines and then either slept or stayed awake on the night of vaccination, post-vaccination sleep boosted immunological memory responses compared with staying awake; the number of antigen-specific Th cells increased, and the fraction of Th1 cytokine-producing cells increased when they slept after vaccination. Sleep also significantly increased the antigen-specific IgG1 levels. One study reported that 24 students in their early 20s were administered influenza vaccine in the morning; 13 had their usual sleep in the evening, and 11 stayed awake until 6 PM the next day [30]. Blood was drawn from the participants on days 5, 10, 17, and 52 to measure the hemagglutination-inhibition titers against the H1N1 virus. A decrease in the serum antibody titer was observed on day 5 post-vaccination in men who could not sleep after vaccination compared with that in those who slept normally. However, there was no difference in the antibody titers between the two groups among women. In addition, there was no difference in the antibody titers between the two groups on days 10, 17, and 52 after vaccination, except on day 5 after vaccination. In this previous study, temporary sleep deprivation was not expected to have a lasting effect on the formation of antibodies against influenza vaccine. Further studies are needed to understand why the effects of sleep deprivation on antibody formation differ between men and women. Prather et al. [31] administered hepatitis B vaccine three times to healthy adults aged 40–60 years to determine whether sleep time affects the production of antibodies to the vaccine antigen. They used actigraphy and electronic sleep diaries to measure the sleep

Table 1. Studies on the relationship between sleep and the immunogenicity of vaccines

First author (year)	Vaccine	Mean age (yr)	No. of participants (% of men)	Study design	Measurements	Outcomes
Spiegel (2002) [26]	Influenza vaccine	23	25 (100)	Sleep restriction (n=11) Normal sleep time (n=14)	Antibody titer 10 days and 3–4 weeks after vaccination	The average antibody titer 10 days after vaccination in the sleep-deprived group was less than half of that in the normal sleep group. There was no significant difference in the antibody titer between the two groups 3–4 weeks after vaccination.
Lange (2003) [28]	HAV	Range: 20–35	19 (47)	Sleep deprivation on the night of vaccination (n=9) Normal sleep (n=10)	Antibody titer 28 days after vaccination	The antibody titers were almost twice as high in those who normally slept after vaccination compared with the titers of those who were awake the night after vaccination.
Lange (2011) [29]	HAV and HBV	26.1	27 (100)	Sleep deprivation on the night of vaccination (n=14) Normal sleep (n=13)	Ag-specific Th cell response and HAV- and HBs-specific Ab	Post-vaccination sleep boosted the development of the Th1 immune response. Sleep enhanced the HAV and HBV-specific IgG1 response.
Benedict (2012) [30]	Influenza A and H1N1 virus vaccine	Sleep deprivation group: 20.4 Sleep group: 20.6	24 (46)	Sleep deprivation (n=11) Normal sleep (n=13)	Specific antibody titer on days 5, 10, 17, and 52 following vaccination	In comparison to the sleep group, the sleep-deprived male group but not the female group had reduced antibody titers 5 days after vaccination. There was no difference in antibody titers at later time points between the sleep group and the sleep-deprived group.
Prather (2012) [31]	HBV	50.1	125 (44)	No intervention	(1) Sleep efficiency (actigraphy-derived), sleep duration (diary-based), or subjective sleep quality	A shorter sleep duration was associated with a lower antibody response to vaccination.
					(2) Blood sample collection: before the second and third vaccinations and 6 months after the last dose	Sleep efficiency and sleep quality were generally not related to the magnitude of the antibody response to HBV.
Prather (2021) [27]	Influenza vaccine	18.3	83 (44)	13 Days of sleep diaries and administration of influenza vaccine on day 3 of the study	Antibodies to the A/New Caledonia viral strain 1 and 4 months after vaccination	A shorter sleep duration on the two nights before the vaccination was associated with fewer antibodies 1 and 4 months after vaccination.

HAV, hepatitis A vaccine; HBV, hepatitis B vaccine; HBs-specific Ab, hepatitis B surface antigen-specific antibody.

time. Actigraphy was used to measure sleep time and sleep efficiency for 6 consecutive days, 3 days before and 3 days after the first vaccination. A shorter sleep duration resulted in lower secondary antibody response. Shorter sleep was also associated with a lower rate of reaching an antibody titer that would be clinically protective against hepatitis B after completion of the third vaccination.

Considering the results of several studies on the relationship between antibody formation for specific antigens after vaccination and sleep, lesser sleep times or sleep deprivation before and after vaccination negatively affected the immunogenicity of vaccines, except for one study that showed no differences in the antibody titer regardless of sleep deprivation in women [30].

Association between circadian rhythms and the immunogenicity of vaccines

Shift workers are an essential group in studies on sleep and circadian rhythms and their relationship to immunity. Therefore, studies have been conducted on changes in immune cells and responses to vaccines in shift workers. In a study of Norwegian nurses, the authors aimed to evaluate differences in immunological biomarkers depending on the work schedule, sleep time, and shift work [32]. However, the work schedule, the number of night shifts, a short sleep duration, and a poor sleep quality did not affect the levels of immunological biomarkers. Therefore, it was not possible to conclude on how shift work affected the immunity in their study. However, when the biomarker levels were compared after a night of sleep with those after a night or day shift, the interleukin (IL)-1 beta levels were higher both after a night shift and day shift than after a night of sleep. The TNF-α levels were higher after a day shift than after a night of sleep. The monocyte chemoattractant protein 1 levels were lower after a night shift and day shift than after a night of sleep. These findings suggest that shift work can acutely affect the immune function. In a study that simulated a 4-day night shift protocol and evaluated its effect on circadian regulation of the human transcriptome, peripheral blood mononuclear cells were collected through two blood sample collections [33]. In the night shift condition, rhythmic transcripts were significantly reduced compared with the baseline. Upon further analysis, the rhythmicity was not completely lost owing to the night shift condition; rather, the rhythm was dampened. Functional analysis showed that the immune response mediated by NK cells and the Jun/AP1 and STAT pathways were mainly affected by night shift work.

A recent study examined 34 workers aged 22–59 years who worked night or day shifts to determine whether night shifts affected the immune responses after vaccination [34]. After the polysomnography test, meningococcal C meningitis vaccine was administered to the participants the following day. The researchers evaluated humoral and cellular immune responses after vaccination. In night shift workers, the levels of inflammatory mediators TNF-α and IL-6 increased before vaccination. In addition, the antibody titers measured on days 28 and 56 of vaccination in night shift workers were significantly lower than those in day workers. The CD4+ T cell count, plasmacytoid DC count, and prolactin level decreased among night shifters, and the Treg cell count increased in those with low antibody titers. Alterations in sleep and circadian rhythms were also associated with decreased antibody responses. The post-vaccination humoral response decreased when night shift-related phase delay was prolonged.

Researchers continue to argue whether shift workers are at an increased risk of developing cancer, cardiovascular disease, metabolic syndrome, and diabetes [35]. Disturbance of circadian rhythms owing to shift work seems likely to affect immune cells and responses, and the effects may consequently cause changes in the immune response after vaccination.

Immunogenicity of vaccines according to the vaccination time during the day

Because the immune system is known to have a circadian rhythm, there have also been studies on the relationship between the vaccine administration time and immunogenicity of vaccines [36,37]. Table 2 presents studies on the relationship between the vaccination time and immunogenicity of vaccines. In the study by Long et al. [38] conducted in the UK, 276 adults aged ≥65 years were divided into two groups: one vaccinated with influenza vaccine in the morning (9–11 AM) and the other group in the afternoon (3–5 PM). The antibody titers against influenza A (H1N1) and B strains were higher in the morning immunization group than in the afternoon immunization group. The antibody

Table 2. Studies on the effects of the vaccine administration time on the immunogenicity of vaccines

First author (year)	Vaccine	Age (yr)	No of participants (% of women)	Study design	Measurement	Outcome
Long (2016) [38]	Influenza vaccine	≥65	276 (49)	Morning vaccination: 9–11 AM Afternoon vaccination: 3–5 PM	(1) Antibody titers: pre- and post- (1 month) vaccination (2) Serum cytokine and steroid hormone levels at baseline	The participants vaccinated in the morning had a greater antibody response than those vaccinated in the afternoon. The cytokine and steroid hormone levels were not related to the antibody responses.
Karabay (2008) [39]	Hepatitis B vaccine	19–23	63 (57)	Morning vaccination (n=30) Evening vaccination (n=33)	Anti-HBs titers: 1 month after the final vaccination	After three doses of vaccine, there was no difference in the geometric mean antibody titers between the morning vaccination group and the evening vaccination group.
Phillips (2008) [40]	Study 1: hepatitis A vaccine Study 2: influenza vaccine	Study 1: mean age of 22.9 Study 2: mean age of 73.1	Study 1: 75 (55) Study 2: 89 (57)	Study 1: morning (n=39) or evening (n=36) vaccination Study 2: time of day of vaccination-an opportunistic variable. A binary AM (n=59)/PM (n=30) variable was created.	Antibody titers: pre- and post- (1 month) vaccination	Men, but not women, vaccinated in the morning showed a stronger antibody response to HAV and influenza vaccines than men vaccinated in the afternoon.
Gottlob (2019) [41]	Hexavalent vaccine	Gestational age at birth: 26–30 weeks	26 (58)	Morning vaccination (n=12) Evening vaccination (n=14)	(1) CER: episodes of hypoxemia or bradycardia (2) Antibody titers for pertussis and *Haemophilus influenzae* type b at a corrected age of 3 months	There was no impact of morning or evening vaccination on the CER. Vaccination led to an increase in the CER in both groups; however, there was no difference in the CER between the morning and evening groups. The antibody titers for *Bordetella pertussis* increased in both groups, with no difference in the levels of inflammatory markers 24 hours after vaccination.

Anti-HBs, hepatitis B surface antibody titers; HAV, hepatitis A vaccine; CER, cardiorespiratory event rate.

response to influenza vaccination in elderly people is a crucial issue but was impressive in this previous study, because it showed the possibility of increasing the effects of influenza vaccination in this population. The antibody response can be increased by simply setting the vaccination time in the morning.

Karabay et al. [39] subdivided 65 participants aged 19–23 years into two groups: one group received hepatitis B vaccine in the morning and the other group in the afternoon. There was no difference in the immune responses between the two groups.

Phillips et al. [40] performed two studies on the vaccine administration time and antibody responses. In their first study on hepatitis A vaccine in 75 students (mean age, 22.9 years), 39 students were vaccinated in the morning and 36 in the evening. In their second study on influenza vaccine in 99 elderly people (mean age, 73.1 years), 59 were vaccinated between 8 and 11 AM and 30 between 1 and 4 PM. The antibody responses to hepatitis A and A/Panama influenza strains were higher in men vaccinated in the morning than in those vaccinated in the evening. However, this was not observed in women. Further studies are needed to determine the reasons for the different antibody responses by sex.

Gottlob et al. [41] observed whether morning or evening vaccination of neonates affected the cardiorespiratory event

rate. They administered the first hexavalent primary vaccine to neonates with 26–30 weeks of gestational age in the morning or evening, counting the episodes of hypoxemia and bradycardia. In addition, they measured the antibody titers against vaccine antigens in each group. There was no difference in the cardiorespiratory event rate between the morning and evening groups, and the antibody titer to *Bordetella pertussis* increased from baseline in both groups; however, a significant increase in the antibody titer to *Haemophilus influenzae* type b (Hib) was observed only in the evening group. However, since the antibody response to Hib was lower in premature babies than in full-term infants in previous studies, one of the reasons for the lower antibody response to Hib in the babies participating in their study may be that the study group included premature babies. The investigators also compared pre- and post-vaccination antibody levels within groups but did not compare the antibody titers between the morning and evening groups. Therefore, based on these results, morning or evening vaccination in premature infants is considered to have no significant effect on the antibody titer or cardiorespiratory event rate. However, the results should be accepted because newborns spend most of their days sleeping, and it is difficult to determine whether they have a circadian rhythm set as in adults.

Impact of circadian rhythms and sleep on the immunogenicity of COVID-19 vaccines

As people worldwide become eligible for vaccination owing to the COVID-19 pandemic, there is increasing interest in the immunogenicity and safety of vaccines. Based on the results of previous studies on the relationship between sleep or vaccine administration time and vaccine efficacy, there were opinions that these factors should be considered in clinical studies on the efficacy of the COVID-19 vaccine [42,43]. In a study of 2,784 healthcare workers in the UK, they received either the Pfizer or AstraZeneca COVID-19 vaccine [8]. There were three categories of vaccination time: time 1, 7:00-10:59 AM; time 2, 11:00 AM–14:59 PM; and time 3, 15:00-21:59 PM. The antibody titers were higher in those vaccinated later when the time 1 group was compared with the time 2 and 3 groups. In this previous study, there were limitations to the interpretation of the results because there was no information on the participants' medical history, sleeping hours, and shift work patterns that could affect the immunogenicity of the vaccines. However, in the study by Zhang et al. [44], 63 medical personnel were divided into 33 and 30 groups, respectively, and administration of an inactivated severe acute respiratory syndrome coronavirus-2

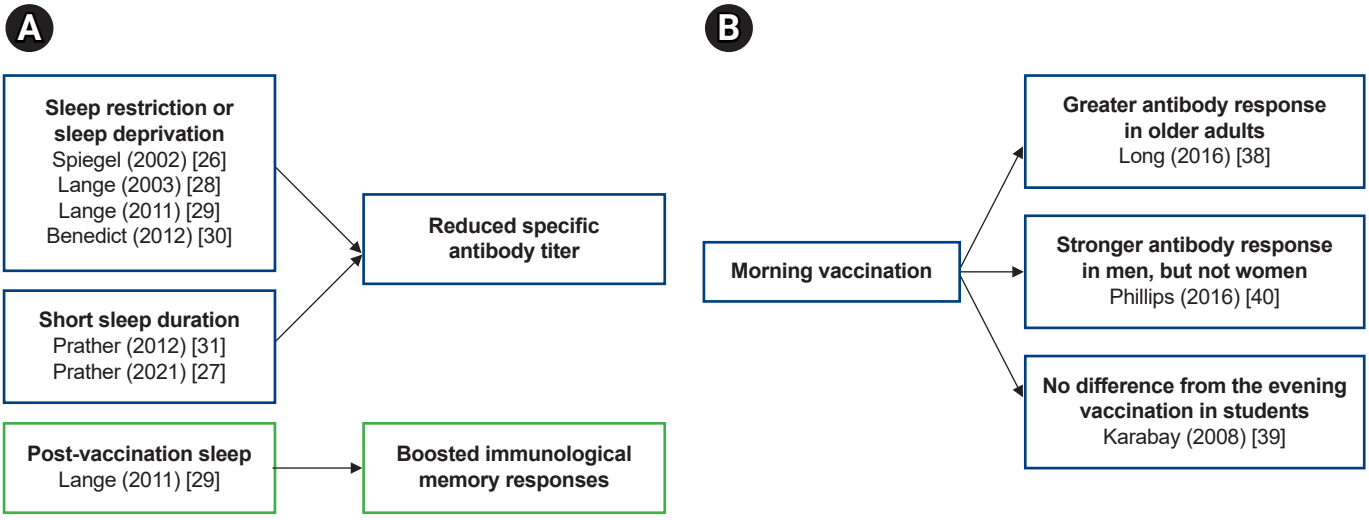

Fig. 1. Summary of study results on the relationship between sleep or vaccine administration time and vaccine efficacy. (A) Sleep deprivation, sleep restriction, or short sleep reduced the antibody response to the vaccine antigen. Sleep boosted the Th1 immune response after immunization. (B) Studies on vaccine administration time and vaccine efficacy differed in study design, and the results on vaccine efficacy were inconsistent.

vaccine (BBIBP-CorV; Sinopharm, Beijing, China) in the morning (9–11 AM) and afternoon (3–5 PM) showed opposite results. Those vaccinated in the morning had stronger B and follicular helper T cell responses than those vaccinated in the afternoon. In addition, those vaccinated in the morning had significantly higher serum neutralizing antibody titers than those vaccinated in the afternoon. In a British study, the antibody titer was high in the afternoon group, while in a Chinese study, the antibody titer was high in the morning group. These two studies administered different types of vaccines and had different study designs; therefore, direct comparison of the results is limited.

A study on the relationship between the immune response to COVID-19 vaccines and sleep and shift work is ongoing [9]. The researchers noted that in previous influenza and hepatitis vaccine studies, sleep deprivation adversely affected the immunogenicity of the vaccines. Therefore, they hypothesized that there might be differences in the antibody response between shift workers and day workers, as circadian rhythm disturbances and sleep deprivation in shift workers could negatively affect their antibody responses to COVID-19 vaccines.

Conclusions

In studies of the relationship between sleep and vaccine immunogenicity, sleep restriction, sleep deprivation, or less sleep reduced antibody responses to vaccine antigens (Fig 1A). Conversely, sleep after vaccination boosted the immune memory response. Considering the effect of the circadian rhythm on the immune response, it is an interesting topic, presumably related to the time of vaccination and the efficacy of the vaccine. However, there have been few studies on this issue so far, and since the study designs were different, it is difficult to draw consistent conclusions from these study results (Fig 1B). In particular, in the relationship between vaccine administration time and vaccine efficacy, the results according to gender and age were different, and additional research is needed on this. First of all, it should be investigated whether the vaccine administration time really affects the vaccine efficacy, and if so, what mechanism works to make it happen. In addition to the antibody response, studies on sleep and vaccine efficacy should be added to cell-mediated immunity.

The two most important factors when applying a new vaccine to a specific population are safety and efficacy based on immunogenicity. We can infer from studies on the effects of sleep and circadian rhythms on immune cells and mechanisms that they can also affect the immunogenicity of vaccines; many researchers have already conducted studies to prove this. It is necessary to further understand the role of sleep and vaccine administration time in the adaptive immune responses that directly affect the immunogenicity of vaccines. Through such, it will be possible to determine the appropriate time to administer vaccines and whether intervention for shift workers is necessary when vaccinated.

Article information

Conflicts of interest

Eun Seok Kim and Chi Eun Oh are editorial board members of the journal but were not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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The effects of ketamine on pain control in stage IV cancer patients receiving palliative care

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Background: We evaluated the effects of intravenous ketamine on cancer pain in stage IV cancer patients receiving palliative care.
Methods: In total, 253 stage IV cancer patients with cancer pain hospitalized at a single tertiary hospital palliative care unit were included. The ketamine group contained 112 patients receiving ketamine, and the control group comprised 141 non-ketamine users. To evaluate the odds ratios (ORs) for favorable pain control, optimal pain control, and opioid-sparing effect among ketamine users, we used multivariable logistic regression adjusted for age and objective prognosis score. Differences in the visual analog scale (VAS) score, oral morphine equivalents, inter-dose frequency, and inter-dose amount were compared between both groups at the time of ketamine introduction (T0), after 24 hours (T1), and after 48 hours (T2) using repeated-measures analysis of covariance.
Results: The ketamine group was more likely to show favorable pain control (OR, 3.84; 95% confidence interval [CI], 1.76–8.37) and an optimal response (OR, 3.99; 95% CI, 1.73–9.22) than the control group. Compared to the control group, the ketamine group showed a higher VAS score at T0, but a more evident VAS score reduction at T1 and T2 ($p_{interaction}$ <0.001). The ketamine group was less likely than the control group to experience depressive mood (OR, 0.31; 95% CI, 0.10–0.92), but had a higher risk of delirium (OR, 2.06; 95% 1.12–3.81).
Conclusion: Our findings suggest that ketamine can effectively reduce refractory cancer pain in stage IV cancer patients.

Keywords: Adverse effects; Cancer pain; Ketamine; Palliative care

Introduction

The prevalence of cancer pain is estimated to be 50.7% in patients with cancer [1] and 70% in patients who have terminal cancer [2]. Refractory cancer pain refers to cancer-related pain that persists over time despite adequate administration of pain control medications, including morphine, and co-analgesics [3]. Conventional treatment cannot adequately relieve cancer pain in 20% to 40% of cases [4,5], and treatment of cancer pain can be challenging due to its complicated pathophysiology [6].

Ketamine was mainly used as an anesthetic agent since the 1970s, but it began to gain interest as an effective analgesic when the N-methyl-D-aspartate (NMDA) receptor was discovered in the 1990s [6]. Ketamine blocks the NMDA receptor non-competitively, and analgesic effect is mediated by antagonism of the NMDA receptor [6,7]. Ketamine has gained interest in research fields because low dose ketamine may decrease the amount of opioid consumption and potentiate opioid activity [8,9]. Previous studies

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regarding the effects of ketamine on cancer pain showed conflicting results. A study from Hong Kong reported that ketamine use decreased the numerical rating scale score for pain from 7 to 4 [10]. Another study from China reported that the intensity of refractory cancer pain was decreased by half among 47% of patients with terminal cancer who used ketamine [10]. Furthermore, ketamine demonstrated superior pain relief effects in peripheral neuropathy compared to lidocaine or placebo [11]. Use of ketamine also showed beneficial effects on various types of pain including chronic and neuropathic pain [11,12]. However, a randomized controlled study conducted in Australia found that ketamine had no additional clinical effect on cancer pain [13].

Although several studies on ketamine have been conducted, there is still a debate regarding the effectiveness of ketamine in the management of cancer pain. Although a randomized controlled study of Australia reported null effect of ketamine on controlling cancer pain, a majority of study participants were composed of lung and prostate cancer patients. Therefore, it is an area of uncertainty whether ketamine has a pain relief effect in terminal stomach and pancreatic cancer which are prevalent in Korea. In addition, despites a modest volume of evidence indicating ketamine might be effective in controlling terminal cancer pain in Asian population, few studies have estimated the pain relief effects of ketamine among patients with terminal cancer in Korea. Therefore, we aimed to evaluate the effect of intravenous ketamine on cancer pain and opioid-sparing among stage IV cancer patients at a palliative care unit.

Methods

All study protocols complied with the Declaration of Helsinki. This study was reviewed and approved by the Institutional Review Board of Kosin University Medical School (KUGH-2020-03-041), and the requirement of informed consent was waived.

1. Participants and study design

We retrospectively assessed 343 stage IV cancer patients with cancer pain who had been hospitalized from January 2015 to February 2019 at a single tertiary hospital palliative care unit. Patients who died within 48 hours after using ketamine, or had missing data on ketamine use or cancer-related variables (n=90) were excluded. Thus, a total of 253 patients were included in the study analysis. Of the 253 patients, we categorized 112 patients receiving ketamine into a ketamine group and 141 non-ketamine users into a control group. We defined stage IV cancer patients as patients who had been diagnosed clinically or histopathology with cancer and showed evidence of distant metastasis.

As the general pain control protocol for our palliative unit was administration of intravenous morphine, patients who were already taking oral opioids were administered intravenous morphine equivalent to one-third of the oral dose. For patients who started opioid therapy at the palliative care unit for the first time, the starting dose of intravenous morphine was 10 mg, and the daily dose was gradually increased until cancer pain was controlled with an inter-dose for ≤4 times a day. An inter-dose of intravenous morphine was one-fifth of the regular-opioid dose and was administered when cancer pain was not relieved with the regular opioid dose. The minimum interval between administrations was 2 hours, but there were no other limits.

Patients with unrelieved cancer pain despite an administration of rapid-acting morphine (20% of regular regular-opioid dose) and dose escalation of regular opioid (50%–100% increment of previous regular opioid dose) were selected for the ketamine group, and 0.5–1 mg/kg/day of ketamine was given.

2. Data collection and measurements

Data on the general characteristics and cancer-related variables such as the time since cancer diagnosis, cancer stage, and occurrence of metastasis were gathered from patients' electronic medical records by two trained medical data managers. A supervisor then reviewed and validated the data. Cancer stage was classified according to the American Joint Committee on Cancer staging system, 7th edition.

Data were collected for 3 days from the first use of ketamine. Each case report contained information on general characteristics, such as anthropometric measurement, and information about the cancer type, disease duration, pain characteristics, and adverse effects profiles. Two independent reviewers verified the information against the medical records. Pain levels were routinely measured by trained medical personnel using the visual analog scale (VAS). The morphine doses used in the study are expressed as the oral morphine equivalent (OME). The conversion ratio of

various morphine equivalents was as follows: oral morphine 100=intravenous morphine 33=transdermal fentanyl 1=intravenous fentanyl 1=oral methadone 20=intravenous methadone 16=oral oxycodone 70=transdermal buprenorphine 1.3 [14]. Data regarding ketamine, co-analgesics, and sedation drug prescriptions were gathered and dichotomously categorized into two groups (yes or no). Body mass index was calculated as weight in kilograms and height in meters (kg/m^2) and divided into three groups according to criteria defined for East Asian populations (<23, 23–24.9, and ≥25 kg/m^2) [15]. Smoking status was categorized into two groups (smokers or non-smokers), and alcohol drinking status was categorized into two groups (yes or no).

The objective prognosis score (OPS) is a tool for predicting the survival of terminal cancer patients in palliative care units in Korea that was developed by Jho et al. [16], and it ranges from 0 to 8 points. In a study of terminal cancer patients admitted to palliative care units in Korea, Jho et al. [16] reported that the sensitivity, specificity, and overall accuracy of OPS with 3 as the cutoff to predict the 3-week survival rates were 74.7%, 76.5%, and 75.5%, respectively. The OPS was assessed by palliative care medical personnel on the first day of admission.

3. Study outcomes

The change in pain intensity, OME, and inter-dose frequency at the time of ketamine introduction (T0), after 24 hours (T1), and after 48 hours (T2) were serially assessed. The primary study outcomes were the change in pain level between T0 and T2. A favorable response and optimal response to ketamine were considered when the pain intensity decreased by ≥2 points and ≥50%, respectively, at 48 hours after the introduction of ketamine [17].

The secondary study outcomes were a reduction in OME, inter-dose frequency, and inter-dose amounts. Since there is no defined dose cutoff for opioid-sparing [18], we arbitrarily defined opioid-sparing effect as a reduction in opioid dose due to the use of ketamine. No opioid-sparing effect was considered when the dose was maintained.

Headache, constipation, micturition associated difficulties, nausea, depressive mood, anxiety, delirium, and dyspnea were defined as ketamine-associated adverse events. After ketamine administration, physicians regularly visited the patients to assess the adverse events via face-to-face interviews for 48 hours. Once patients were compliant with depression, patient health questionnaire-9 (PHQ-9) was used to evaluate depressive mood, and patients ≥10 points in PHQ-9 were determined having depressive mood.

4. Statistical analysis

The general characteristics and cancer-related variables of ketamine users and non-users were compared using a *t*-test for continuous variables and chi-square test for categorical variables. To evaluated the odds ratio (OR) for favorable pain control, optimal pain control, and opioid-sparing effect among ketamine users, we used a multivariable logistic regression analysis that was adjusted for age and OPS. Differences in VAS score, OME, inter-dose frequency, and inter-dose amount were compared between the ketamine group and control group at T0, T1, and T2 using repeated analysis of covariance.

All tests were two-tailed, and a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA).

Results

1. Clinical characteristics

The general characteristics of the study participants are presented in Table 1. Two hundred and fifty-three patients (145 males and 108 females) were included. The mean age of the control group (67.4 years) was higher than that of the ketamine group (61.5 years). The most prevalent cancer type was lung cancer, followed by stomach and pancreatic cancer. The mean length of hospital stay of the ketamine group and control group was 23.2 days and 24 days, respectively. The ketamine group had a higher OPS than did the control group, and the OPS ≥2 was 62% in the ketamine group and 46.1% in the control group. The ketamine group was more likely to use co-analgesic (39.3%) and sedative drugs (70.5%) than was the control group (5% and 34.8%, respectively). Visceral pain was the most prevalent type of pain, followed by musculoskeletal and somatic pain. There were no significant differences in sex, body mass index, cancer diagnosis, time since cancer diagnosis, smoking status, alcohol drinking status, length of hospital stay, sleep time, or types of opioid between the two groups.

Table 1. General characteristics of the study participants

Characteristics	Total (n=253)	Ketamine group (n=112)	Control group (n=141)	*p*-value
Sex				0.665
Male	145 (57.3)	62 (55.4)	83 (58.9)	
Female	108 (42.7)	50 (44.6)	58 (41.1)	
Age (yr)	64.8±11.6	61.5±11.3	67.4±11.2	<0.001
Body mass index[a]				0.656
<23 kg/m^2	103 (40.7)	48 (42.9)	55 (39.0)	
23–24.9 kg/m^2	23 (9.1)	15 (13.4)	8 (5.7)	
≥25 kg/m^2	11 (4.3)	5 (4.5)	6 (4.3)	
Data not provided	116 (45.8)	44 (39.3)	72 (51.1)	
Diagnosis[b]				0.886
Lung cancer	40 (15.8)	11 (9.8)	29 (20.6)	
Stomach cancer	36 (14.2)	15 (13.4)	21 (14.9)	
Pancreatic cancer	34 (13.4)	21 (18.8)	13 (9.2)	
Rectal cancer	23 (9.1)	12 (10.7)	11 (7.8)	
Hepatocellular carcinoma	20 (7.9)	5 (4.5)	15 (10.6)	
Others	100 (39.5)	48 (42.9)	52 (36.9)	
Time since cancer diagnosis				0.768
<1 yr	101 (39.9)	44 (39.3)	57 (40.4)	
1–3 yr	83 (32.8)	37 (33.0)	46 (32.6)	
≥3 yr	69 (27.3)	31 (27.7)	38 (27.0)	
Smoking status				0.821
Smoker	47 (18.6)	22 (19.6)	25 (17.7)	
Non-smoker	206 (81.4)	90 (80.4)	116 (82.3)	
Alcohol consumption				0.848
Yes	45 (17.8)	21 (18.8)	24 (17.0)	
No	208 (82.2)	91 (81.2)	117 (83.0)	
Length of hospital stay (day)	23.7±43.8	23.2±38.7	24.0±47.7	0.537
Objective prognosis score[c]				0.009
0	20 (7.9)	3 (2.7)	17 (12.1)	
1	99 (39.1)	40 (35.7)	59 (41.8)	
2	101 (39.9)	53 (47.3)	48 (34.0)	
≥3	33 (13.0)	16 (14.3)	17 (12.1)	
Co-analgesic (Keromin) use				<0.001
Yes	51 (20.2)	44 (39.3)	7 (5.0)	
No	202 (79.8)	68 (60.7)	134 (95.0)	
Sedation drug use				<0.001
Yes	128 (50.6)	79 (70.5)	49 (34.8)	
No	125 (49.4)	33 (29.5)	92 (65.2)	
Sleep time (hr)	6.0±0.7	6.0±0.7	6.0±0.7	0.954
Type of pain				0.030
Somatic	1 (0.4)	0	1 (0.8)	
Visceral	245 (96.8)	112 (100)	133 (94.3)	
Musculoskeletal	7 (2.8)	0	7 (5.0)	
Type of opioid				0.074
Intravenous	237 (93.7)	103 (92.0)	134 (95.0)	
Patch	5 (2.0)	0	5 (3.5)	
Intrathecal	11 (4.3)	9 (8.0)	2 (1.4)	

Values are presented as number (%) or mean±standard deviation.
p-values were calculated using the *t*-test for continuous variables and the chi-square test for categorical variables.
[a]Body mass index was categorized based on criteria tailored for East Asian populations.
[b]The stage of cancer was IV and was categorized according to the American Joint Committee on Cancer staging system, seventh edition.
[c]The objective prognosis score is a tool for predicting the survival of terminal cancer patients in palliative care units in Korea that was developed by Jho et al. [16], and it ranges from 0 to 8 points.

2. Efficacy: pain control

The OR for favorable pain control, optimal pain control, and opioid-sparing effect are displayed in Table 2. While the ketamine group showed more favorable (OR, 3.84; 95% confidence interval [CI], 1.76–8.37) and optimal responses (OR, 3.99; 95% CI, 1.73–9.22) compared to the control group, there was no significant difference in opioid-sparing effect between the two groups.

Changes in VAS score, OME, inter-dose frequency, and inter-dose amount are presented in Fig. 1.

Although the VAS score of the ketamine group was higher than that of the control group at T0, the degree of VAS score reduction was more evident in the ketamine group than in the control group both at T1 and T2. However, OME, inter-dose frequency, and inter-dose amount was not significantly different between the ketamine group and the control group.

3. Tolerance: adverse effects

Adverse effect profiles associated with ketamine use are presented in Table 3. The most common adverse event in the ketamine group was dyspnea (n=90), followed by

Table 2. Comparison of favorable responses, optimal responses, and opioid-sparing effect between the ketamine and control groups

	Control group (n=141)		Ketamine group (n=112)		*p*-value
	No. (%)	OR	No. (%)	OR (95% CI)	
Favorable response[a]	11 (7.8)	Reference	28 (25.0)	3.84 (1.76–8.37)	0.001
Optimal response[b]	9 (6.4)	Reference	25 (22.3)	3.99 (1.73–9.22)	0.001
Opioid-sparing effect[c]	76 (53.9)	Reference	52 (46.4)	0.64 (0.38–1.09)	0.101

OR, odds ratio; CI, confidence interval.
p-values were calculated using a multivariable logistic regression after adjusting for age and the objective prognosis score.
[a]A favorable response to ketamine was defined as a reduction in pain intensity by ≥2 points at 48 hours after the introduction of ketamine.
[b]An optimal response was defined as a reduction in the pain intensity by ≥50% at 48 hours after the introduction of ketamine.
[c]Since there is no dose cutoff for opioid-sparing, we arbitrarily defined an opioid-sparing effect as a reduction of the opioid dose due to ketamine.

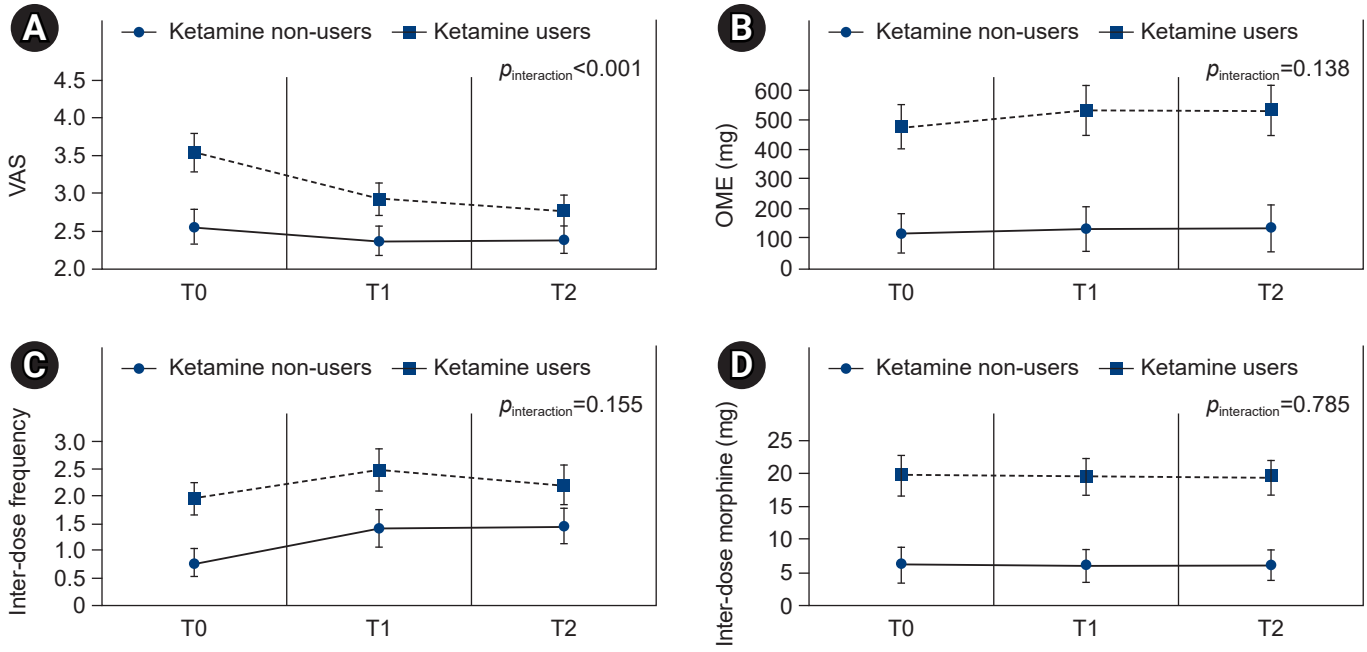

Fig. 1. Changes in VAS score, OME, inter-dose frequency, and inter-dose amount over time in the two groups: T0, ketamine or placebo introduction time; T1, 24 hours after treatment initiation; and T2, 48 hours after treatment initiation. (A) Change in VAS score after ketamine initiation. (B) Change in OME after ketamine use. (C) Change in inter-dose frequency after ketamine use. (D) Change in inter-dose amount after ketamine use. VAS, visual analog scale; OME, oral morphine equivalent.

Table 3. Prevalence of and odds ratios for adverse events among ketamine users

Adverse events	Control group (n=141)	Ketamine group (n=112)	*p*-value
Headache			
Prevalence	9 (6.4)	7 (6.3)	0.966
OR (95% CI)	Reference	1.13 (0.39–3.30)	0.824
Constipation			
Prevalence	23 (16.3)	19 (17.0)	0.890
OR (95% CI)	Reference	1.05 (0.52–2.10)	0.895
Difficulty urinating			
Prevalence	22 (15.6)	22 (19.6)	0.400
OR (95% CI)	Reference	1.34 (0.68–2.65)	0.402
Nausea			
Prevalence	30 (21.3)	34 (30.4)	0.099
OR (95% CI)	Reference	1.46 (0.80–2.65)	0.220
Depressive mood			
Prevalence	15 (10.6)	5 (4.5)	0.071
OR (95% CI)	Reference	0.31 (0.10–0.92)	0.034
Anxiety			
Prevalence	15 (10.6)	18 (16.1)	0.202
OR (95% CI)	Reference	1.72 (0.79–3.71)	0.171
Delirium			
Prevalence	29 (20.6)	35 (31.3)	0.052
OR (95% CI)	Reference	2.06 (1.12–3.81)	0.021
Dyspnea			
Prevalence	49 (34.8)	41 (36.6)	0.759
OR (95% CI)	Reference	1.07 (0.63–1.84)	0.795

Values are presented as number (%) unless otherwise indicated. Adverse effects were observed for 48 hours after ketamine administration.
OR, odds ratio; CI, confidence interval.
p-values were calculated using the chi-square test for categorical variables or multivariable logistic regression for ORs (age and objective prognosis score were adjusted in the multivariable logistic analysis).

nausea (n=68) and delirium (n=64). Participants in the ketamine group were less likely to complain of depressive mood (OR, 0.31; 95% CI, 0.10–0.92) compared to the control group, whereas, the ketamine group showed higher risk for delirium (OR, 2.06; 95% CI, 1.12–3.81). There were no statistically significant differences in other adverse events between the two groups.

Discussion

This retrospective study included 253 patients with terminal cancer pain. Our results suggest that patients who received parenteral ketamine treatment at the palliative care unit showed a decrease in VAS score associated with ketamine use. In addition, the ketamine group had a higher likelihood for favorable and optimal response compared to the control group.

Although the ketamine group was less likely to experience depressive mood, their risk for delirium was higher compared to that of the control group. There was no difference in other adverse events between the two groups.

This study showed that ketamine was associated with decreased VAS score in end-stage cancer patients, and this result was consistent with those of previous studies. Cheung et al. [10] reported that 74.3% of patients (n=52) with refractory cancer pain successfully responded to ketamine. Another randomized, controlled, double-blind study reported that ketamine use reduced refractory cancer pain effectively in most patients [7]. Furthermore, Jackson et al. [2] showed in an open-label study that 67% of patients with refractory cancer pain from four palliative care units successfully responded to ketamine. The beneficial effects of ketamine on controlling cancer pain were also supported by a systemic review arguing that ketamine might be a viable treatment

option for refractory cancer pain [19].

However, the efficacy of ketamine in the management of cancer pain is still controversial. A blinded, randomized, controlled study of 185 adult cancer patients with cancer pain by Hardy et al. [13] reported that ketamine administration over 5 days showed no clinical advantage over placebo with increased adverse events. In addition, an earlier systemic review reported that there is limited evidence on ketamine being effective for controlling refractory cancer pain [20]. A recent systematic review also argued that there is insufficient evidence to conclude on the efficacy of ketamine for patients with refractory cancer pain [21]. The difference in pain control results among the previous studies might be due to different study populations, heterogeneous study settings, and inconsistent outcome definitions.

We found that there was no difference in OME and inter-dose reduction between the two groups; therefore, no opioid-sparing effect was observed with ketamine use in our study. Reduced pain intensity after ketamine administration did not lead to a reduction in the administration of opioids because patients were reluctant to reduce their opioid dose as they were satisfied with their current pain medications. A small sample study reported that patients are more concerned about increasing pain that occurs when opioids are reduced than about the risk of overusing them [22]. Furthermore, based on the Centers for Disease Control and Prevention guidelines, physicians are recommended to reduce opioid dosage by 10% a month, so 48 hours may not have been enough time to observe significant opioid dose reduction [23].

Mercadante et al. [24] reported that ketamine had no opioid-sparing effect, and this finding was in line with our findings. However, several previous studies observed opioid-sparing effects related to ketamine use. Fitzgibbon and Viola [25] found a 25% median opioid dose reduction, and Courade et al. [6] also reported opioid-sparing effects (10.5%). However, the number of participants in these studies and the number of patients showing opioid-sparing effects were small. Therefore, further studies are needed.

We observed that the ketamine was less likely to experience depressive mood than was the control group. In line with our study finding, a recent narrative review reported that ketamine has antidepressant effect with a rapid onset time (within 24 hours) [26]. However, compared to that in the control group, the risk of delirium was elevated in participants who received ketamine. Although several studies have suggested that ketamine use might reduce postoperative delirium [27,28], a study reported that ketamine use might be a possible cause of delirium in cancer patients [29]. Considering the mechanism of action of ketamine, it is plausible to consider safety issues regarding psychological conditions with ketamine use. However, previous studies by Cheung et al. [10] and Mercadante et al. [7] showed tolerable safety profiles of ketamine. In addition, Sheehy et al. [30] investigated the frequency of adverse psychological events, such as hallucinations and changes in sleep patterns, with ketamine use and found no adverse events related to ketamine administration. However, it is still unclear whether the cause of delirium in our patients was the ketamine or cancer itself because delirium is prevalent in patients with terminal cancer.

A few mechanisms can explain the pain control effects of ketamine. A previous study showed that ketamine acted as an antagonist at the NMDA receptor site, which is essential for central sensitization in pain [31]. By blocking this receptor, ketamine could exert its pain control effect [32]. Another possible mechanism is that the combined use of ketamine and morphine has a synergistic effect on pain control that was better than that of either drug alone. In an experimental study, Bossard et al. [9] reported that ketamine and morphine together significantly decreased the threshold of nociceptive flexion reflex compared to morphine or ketamine alone.

This study had several limitations. First, since the study design was retrospective, and VAS scores were measured by medical personnel, VAS scores might be underestimated [33]. However, evaluation and documentation of pain are regularly performed using standardized assessment forms in our palliative care unit to ensure the quality of care. Second, because this study was conducted in stage IV cancer patients, the results of this study cannot be generalized to all cancer patients. Third, while there was consistency regarding the indication of ketamine use, the timing of the administration and ketamine dose could vary.

In conclusion, this study suggests that ketamine can effectively decrease refractory cancer pain in patients with stage IV cancer. Further prospective studies with a larger sample size should be conducted in the future to confirm and expand on these findings.

Article information

Conflicts of interest
Jihun Kang is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Conceptualization: SK, JK. Data curation: SK, JK. Formal analysis: SK, JK. Methodology: SK, JK. Visualization: SK, JK. Writing - original draft: SK, JK. Writing - review & editing: SK, JK, JC, EK. Approval of final manuscript: all authors.

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Comparison of the radiation dose between dual-acquisition coronary computed tomography angiography and coronary angiography for coronary spasm

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Background: Coronary computed tomography angiography (CCTA) is an imaging technique which can be used to evaluate and diagnose coronary artery stenosis. Dual-acquisition CCTA (DA-CCTA) with additional nitrate infusion is a promising alternative noninvasive diagnostic tool, as conventional CCTA has limitations in the diagnosis of variant angina compared to conventional angiographic coronary spasm tests. However, DA-CCTA may pose a potential risk due to radiation exposure. We compared the radiation dose between DA-CCTA and the coronary angiographic spasm provocation test.

Methods: Patients with clinically suspected variant angina at a single hospital between March 2013 and October 2018 were screened and underwent DA-CCTA or a coronary angiographic spasm provocation test. The effective radiation dose required for each approach was compared.

Results: In total, 211 suspected variant angina patients underwent DA-CCTA or the coronary angiographic spasm provocation test. Of these, 49 patients (mean age, 59.8 years; 67.3% men) received DA-CCTA and 162 patients (mean age, 60.5 years; 66.2% men) received a coronary angiographic spasm provocation test. There was meaningful difference in the effective radiation dose, with a median dose of 5.1 mSv (interquartile range [IQR], 4.1–9.2 mSv) required for DA-CCTA and a median dose of 10.9 mSv (IQR, 8.4–15.2 mSv) for the coronary angiographic spasm provocation test (p<0.001).

Conclusion: DA-CCTA showed a significantly lower effective radiation dose than the coronary angiographic spasm provocation test required to diagnose variant angina.

Keywords: Computed tomography; Coronary angiography; Coronary vasospasm; Radiation dosage

Introduction

The frequency of radiation exposure is increasing in the cardiology field as the application of invasive coronary angiography (CAG) and coronary computed tomography angiography (CCTA) increases [1-5]. In particular, the use of CCTA is increasing as CCTA technology continues to advance [6-8]. For the diagnosis of variant angina, the invasive coronary angiographic spasm provocation test remains the gold standard [9,10]. However, CAG is an invasive procedure requiring catheter insertion into the heart and carries a risk of complications including fetal cardiac arrhythmia.

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In comparison to CAG, CCTA holds an advantage in being noninvasive, but has limited diagnostic utility in the diagnosis of variant angina [11]. Previous studies have suggested that dual-acquisition CCTA (DA-CCTA) with nitrate infusion may be suitable for the diagnosis of coronary spasm [12,13]. The benefits of DA-CCTA for noninvasive testing include diagnostic feasibility but concerns regarding the risk of two radiological exposures in DA-CCTA remain. In addition, there is little data on the radiation exposure involved in the invasive coronary angiographic spasm provocation test and DA-CCTA in the diagnosis of variant angina [14,15]. Thus, we sought to evaluate the effective radiation dose for DA-CCTA and the invasive coronary angiographic spasm provocation test in the diagnosis of variant angina and determine whether DA-CCTA would be comparable.

Methods

Ethical statements: This study was approved by the Institutional Ethics Committee of Dong-A University Hospital (DAUHIRB-17-225). The informed consent was waived because this design is a retrospective study.

1. Patient selection

Patients with clinically-suspected variant angina who visited Dong-A University Hospital in Busan, South Korea, between March 2013 and October 2018 were screened, and all of who underwent DA-CCTA or the invasive coronary angiographic spasm provocation test with available radiation dose information were selected. The final analysis included 211 subjects and we retrospectively collected radiation dose information with clinical data including sex, age, body weight, height, and heart rate.

2. Dual-acquisition of CCTA

DA-CCTA consists of baseline CCTA without vasodilator (CCTA step 1) and nitrate CCTA (CCTA step 2). The CCTA step 1 was performed in the early morning without intravenous nitrate infusion. And the CCTA step 2 was followed by nitrate infusion after the CCTA step 1. Administration of any calcium channel blocker or nitrate substrates was stopped over 3 days before the examination, and beta blockers (carvedilol 12.5 mg) were prescribed to patients presenting with a heart rate over 70 beats per minute. For the CCTA step 2, intravenous nitrate was injected at 2 mg/hr prior to 30 minutes of CCTA for effective vasodilation. All CCTAs were implemented using a 320-detector row scanner (Aquilion ONE, Toshiba Medical Systems, Otawara, Japan) with 320×0.5 mm^2 collimation, a 350-millisecond gantry rotation time and 175-millisecond temporal resolution. Tube voltage and current were adjusted using the software (Sure Exposure 3D®, Toshiba Medical Systems). The tube voltage was 120 kVp and the tube current was 130–250 mA. The contrast medium was used as nonionic (iobitridol, Xenetix 350 mgI/mL; Guerbet, Villepinte, France), and 50–60 mL of it was injected intravenously at a rate of 4 mL/s, and then 30 mL of a contrast medium and saline mixture (2:8 dilution) was injected at a rate of 4 mL/s. Automatic bolus trigger system activated computed tomography (CT) scan in the ascending aorta with a delay of 5 seconds. Under sufficiently slow heart rate condition (≤65 beat/min), CT scans were acquired with prospectively triggered data collection at 70%–80% of the RR interval. When the heart rate was relatively rapid (>65 beat/min), CT scans were obtained by retrospective data acquisition with electrocardiography (ECG)-based tube current modulation (full tube current 30%–80%). In the few cases with rapid and irregular heart rates, the images were generated using multisegment reconstructions of the heartbeats according to the default values of the CT scanner. The images were subjected to iterative reconstruction processing reconstruction (AIDR 3D, Toshiba Medical Systems). The images were obtained with a thickness of 0.5 mm and an interval of 0.5 mm, and the axial image was reconstructed according to the heart size of each individual. All images obtained were analyzed using the software (Aquarius iNtuition Edition ver. 4.4.11; TeraRecon Inc., Foster City, CA, USA). Reconstructed images from the 75% cardiac phase or the automatically suggested "best phase (ms)" were used for interpretation. For some cases with rapid heart rates, additional systolic or diastolic phase images were used.

3. Coronary angiographic spasm provocation test

CAG was performed using a Allura Xper FD 10/10 (Philips Medical Systems Nederland B.V, Eindhoven, Netherlands). Coronary angiographic spasm provocation test was consisted of the baseline CAG (CAG step 1) and the ergonovine provocation test (CAG step 2). The CAG step 1 was performed on the left coronary artery and then on the right coronary artery. In the CAG step 2, coronary artery spasms were induced by administration of ergonovine (10–20 μg)

in the coronary artery only if there was less than 50% vessel stenosis. When coronary artery spasms were induced after CAG in the right coronary artery, they were controlled by injecting nitroglycerin into the coronary artery. If coronary artery spasms were not observed following right CAG, an induction test for the left coronary artery was performed. The ergonovine was injected (10–20 μg) into the coronary artery. Each of the left and right coronary arteries was dosed up to 3 times at 1-minute intervals.

The positive determination for the ergonovine provocation test was based on the findings for myocardial ischemia (angina symptom or ischemic ECG changes: transient ST segment elevation, ST segment elevation above 0.1 mV, or transient depression) with total or subtotal occlusion, which temporarily reduced the diameter of the coronary artery by more than 90% (negative U-wave was recorded).

4. Radiation dose estimates

Effective radiation doses are expressed in mSv units, and the effective radiation dose for CCTA was calculated by multiplying the dose-length-product by the European Working Group for Guidelines on Quality Criteria in CT conversion coefficient (k=0.014 [mSv]/[mGy×cm]) [16,17]. For CAG, the effective dose was calculated using a coefficient for converting the dose area product to the effective dose (k=0.18 [mSv]/[Gy×cm^2]) [18].

5. Statistical analysis

Data analysis was performed using statistical analysis software (SPSS version 18; SPSS Inc., Armonk, NY, USA). Continuous variables are expressed as mean±standard deviation or median with interquartile range (25th and 75th percentiles); nominal variables are expressed as frequencies and/or percentage, as appropriate. The Kolmogorov-Smirnov test was used to test whether data are normally distributed and the chi-square test was used to evaluate proportions of categorical data. Systematic differences between the two groups were assessed using independent Student *t*-test or Mann-Whitney *U* test. Differences of effective radiation dose between the CCTA group and the CAG group presented as median/mean value and *p*-value estimated by Mann-Whitney *U* test. In addition, we performed generalized linear model by using age, sex, and body mass index (BMI) and estimated mean (standard error). Differences between the CAG step 1 and the CAG step 2, and differences between CCTA step 1 and CCTA step 2 were evaluated using paired *t*-test or Wilcoxon rank-sum test. Comparison of the radiation doses according to diagnosis was assessed by using one-way analysis of variance test or Kruskal Wallis test. A *p*-value <0.05 was considered statistically significant.

Results

The baseline clinical characteristics of the patients are shown in Table 1. The mean age was 60.4 years old, and 65.4% were male. There were no significant differences in age, sex, or BMI between the two groups.

The effective radiation dose was significantly higher in the CAG group than in the CCTA group (median [IQR]: 10.9 [8.4–15.2] mSv vs. 5.1 [4.1–9.2] mSv, *p*<0.001) (Fig. 1). Moreover, age, sex, BMI adjusted mean value were significantly higher in the CAG group than the CCTA group (mean±standard error: 7.4±1.5 mSv vs. 13.5±0.9 mSv, *p*<0.001) (Table 2).

For the CCTA group, there was no significant difference in the effective radiation dose between the CCTA step 1 and the CCTA step 2 (median [IQR]: 2.5 [2.0–4.5] mSv vs. 2.9 [2.2–4.5] mSv, *p*=0.162). The radiation dose and heart rate data on the CCTA group is summarized in Table 3. In the CAG group, if the two steps are divided into the CAG step 1

Table 1. Baseline characteristics of the study population

Characteristics	CCTA group (n=49)	CAG group (n=162)	*p*-value
Age (yr)	59.8±8.0	60.5±10.8	0.660
Male sex	33 (67.3)	131 (66.2)	0.910
Height (cm)	163.5±8.7	164.6±9.0	0.431
Weight (kg)	64.0±11.0	67.4±12.5	0.101
Body mass index (kg/m^2)	23.9±2.7	24.8±3.5	0.095
Diabetes mellitus	3 (6.1)	34 (21.0)	0.017
Hypertension	18 (36.7)	54 (33.3)	0.660
Dyslipidemia	7 (14.3)	25 (15.4)	0.845
Current smoker	6 (12.2)	18 (11.1)	0.827
Alcohol consumption (>1 drink/day)	4 (8.2)	4 (2.5)	0.087
Chronic kidney disease	0	0	NA
Chronic lung disease	0	1 (0.6)	NA
Previous CAD	11 (22.4)	31 (19.1)	0.611
Previous CVA	1 (2.0)	4 (2.5)	NA
Sinus rhythm	45 (91.8)	161 (99.4)	0.010

Values are presented as mean±standard deviation or number (%).
CCTA, coronary computed tomography angiography; CAG, coronary angiography; CAD, coronary artery disease; CVA, cerebrovascular accident; NA, not available.

and the CAG step 2, there was a significant difference in the dose area product (median [IQR]: 28.1 [19.6–40.5] Gy·cm^2 vs. 32.1 [22.2–44.9] Gy·cm^2, p=0.007) and effective radiation dose (median [IQR]: 5.1 [3.5–7.3] mSv vs. 5.8 [4.0–8.1] mSv, p=0.007) between the two steps (Table 3). In the CAG group, the dose area product was different depending on the results of diagnosis (positive, equivocal, negative: 61.1 [47.3–80.3] Gy·cm^2 vs. 82.1 [59.0–142.7] Gy·cm^2 vs. 54.9 [41.2–86.5] Gy·cm^2, p<0.05; post hoc analysis: equivocal>positive=negative) (Fig. 2). In addition, the dose area product noted more significant differences on the CAG step 2 (positive, equivocal, negative: 32.1 [22.5–43.8] Gy·cm^2 vs. 53.2 [36.7–85.7] Gy·cm^2 vs. 28.0 [15.8–39.7] Gy·cm^2, p=0.002; post hoc analysis: equivocal>positive=negative).

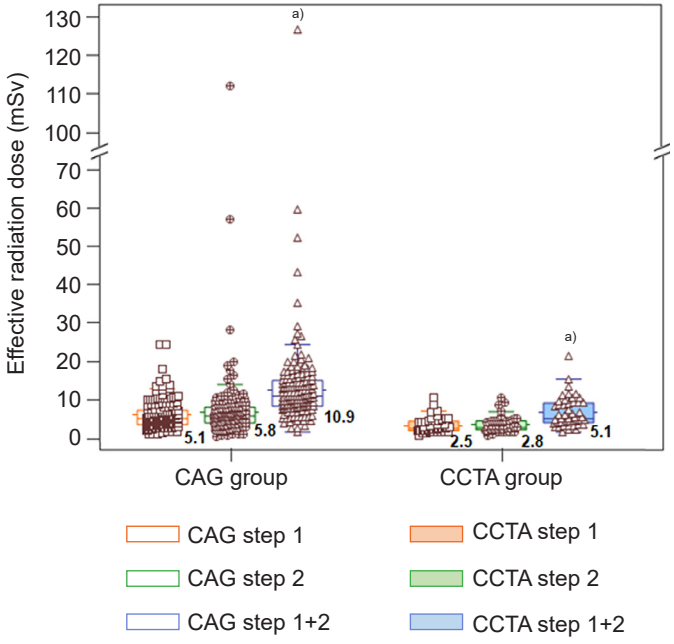

Fig. 1. Comparison of the effective radiation dose between the CAG and CCTA groups. The effective radiation dose was significantly higher in the CAG group than in the CCTA group. Values in the figure are in the median. CCTA, coronary computed tomography angiography; CAG, coronary angiography. [a]p<0.001.

Discussion

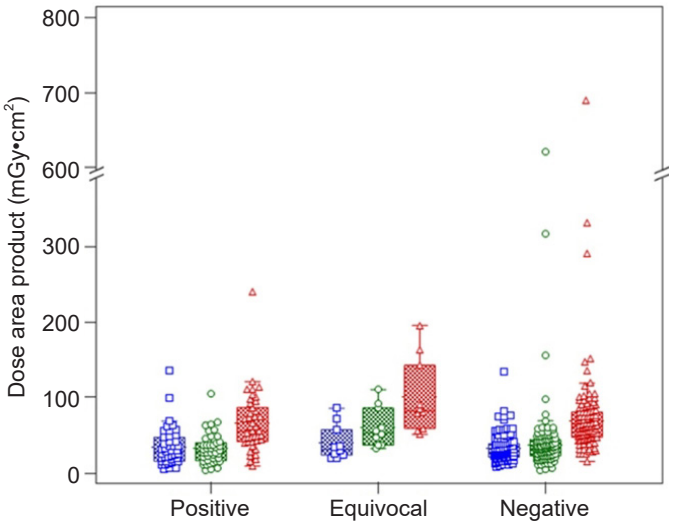

Fig. 2. Differences in radiation exposure according to diagnosis in the coronary angiography (CAG) group. In the CAG group, the dose area product differed according to the results from the ergonovine provocation test (positive, equivocal, negative: 61.1 vs. 82.1 vs. 54.9, p<0.05).

Table 2. Effective radiation dose of the CCTA and CAG groups

Variable	CCTA group (n=49)	CAG group (n=162)	p-value
Effective radiation dose (mSv)			
Median (IQR)	5.1 (4.1–9.2)	10.9 (8.4–15.2)	<0.001
Mean±SD	6.7±3.9	13.3±11.7	<0.001
Mean±SE[a]	7.4±1.5	13.5±0.9	<0.001

CCTA, coronary computed tomography angiography; CAG, coronary angiography; IQR, interquartile range; SD, standard deviation; SE, standard error.
[a]Adjusted mean±SE using age, sex, and body mass index.

Table 3. Radiation dose of each step in the CCTA and CAG groups

Parameter	CCTA group (n=49)			CAG group (n=162)		
	Step 1	Step 2	p-value	Step 1	Step 2	p-value
DLP (mGy·cm^2)	178.4 (141.7–324.4)	209.4 (158.3–323.9)	0.162			
DAP (mGy·cm^2)				28.1 (19.6–40.5)	32.1 (22.2–44.9)	0.007
Effective radiation dose (mSv)	2.5 (2.0–4.5)	2.9 (2.2–4.5)	0.162	5.1 (3.5–7.3)	5.8 (4.0–8.1)	0.007
Heart rate during image acquisition (beats/min)	58.5 (53.5–67.0)	58.5 (53.5–65.0)	0.520			
Heart rate at CAG (beats/min)				72.0 (61.0–81.0)		

Values are presented as median (interquartile range).
CCTA, coronary computed tomography angiography; CAG, coronary angiography; DLP, dose length product; DAP, dose area product.

In this study, the CCTA group required a lower effective radiation dose than the CAG group. In addition, the CAG group showed diverse radiation doses according to the results of the provocation test. Conversely, the CCTA group showed no significant differences in the dual acquisition.

The effective radiation dose for CCTA has been reported as 14.9 mSv and 10.3 mSv in single-source 64 CCTA and dual-source 128 CCTA, respectively [8,14,17,19]. By using a 320-detector row CT scanner, the effective radiation dose can be reduced to a range between 0.58 mSv and 4.0 mSv [19,20]. This study showed a comparable effective radiation dose level for individual CCTA (mean 3.3 mSv) to the recent studies. The radiation dose for CAG has been reported as the dose area product ranging from 23.5 Gy·cm^2 to 55.9 Gy·cm^2, and coronary angioplasty had a higher level than diagnostic CAG (range, 53.8–101.9 Gy·cm^2) [21-23]. In this study, the dose area product for total CAG was 61.4 Gy·cm^2, and the constituents (CAG step 1 and CAG step 2) showed a similar radiation dose. However, the results of the spasm provocation test affected fluoroscopic time and radiation dose, and we can expect diverse results according to the requirements of the individual operator. Moreover, there have been few studies of additive radiation doses according to the spasm provocation test in previous reports, and it was found that the radiation dose was over 2-fold higher for diagnostic CAG or was similar to that for coronary angioplasty. In addition, the spasm provocation test via CAG may carry a risk of lethal arrhythmia. Radiation doses have been lowered and quality images can be achieved along with advanced technology, for example, wide-area detector scanners which can make shorter scanning times, helical oversampling, and using an advanced electrocardiogram-gated reconstruction technique. Our findings suggest that the radiation hazard associated with DA-CCTA is less than that for the coronary angiographic spasm provocation test and DA-CCTA may be a good noninvasive diagnostic option for coronary artery disease and coronary artery spasm.

We acknowledge limitations to this study in that values such as dose length product and dose area product do not allow for comparison between different imaging modalities [14,24]. The radiation dose calculation was performed using a conversion factor from a previous study. However, there are significant variations between previous study results. Additionally, many of the previous studies used 16 detector multi-slice CT [14]. Most of the subjects enrolled in the present study had suitable heart rate and low heart rate variability, although the dose length product of CCTA could be affected by heart rate variability. The patients were not randomized, and the analysis of the data was conducted retrospectively. Furthermore, additional studies such as phantom study for accurate radiation dose comparison was not performed beforehand.

In conclusion, DA-CCTA showed lower the effective radiation dose than the invasive coronary angiographic spasm provocation test in this study, but with limited clarity.

Article information

Conflicts of interest

This study was partly supported by a research grant from Bayer Co. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

Conceptualization: MHK. Data curation: SJK. Formal analysis: SJK. Funding acquisition: MHK. Methodology: MHK, EJK. Project administration: MHK. Visualization: SJK. Writing - original draft: SJK. Writing - review & editing: SJK, MHK, EJK. Approval of final manuscript: all authors.

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Transcriptome analysis of the pathogenic ciliate *Miamiensis avidus* after hydrogen peroxide treatment

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Background: The scuticociliate *Miamiensis avidus* is a highly pathogenic ciliate responsible for serious damage to various organs of aquaculture fish. In particular, the olive flounder aquaculture industry is suffering massive losses due to *M. avidus* infection. Hydrogen peroxide (H_2O_2) is one of the most widely used chemicals for scuticociliate treatment. Despite the superior killing effect of H_2O_2, studies on transcription levels and gene expression changes after H_2O_2 treatment are limited. We conducted an mRNA transcriptome analysis to compare the differentially expressed gene (DEG) profiles between the ciliate and cyst-like stages of *M. avidus* after H_2O_2 treatment.

Methods: We applied DEG profiling to identify DEGs during the ciliate and cyst-like stages of *M. avidus*.

Results: There were 5,967 DEGs among the 9,075 transcripts identified, and 50 of these DEGs were significantly different ($p<0.05$). Among these, 21 DEGs were upregulated and 29 were downregulated in the cyst-like stage. The most significantly upregulated genes during the change to the cyst-like stage were cytochrome c oxidase genes. Genes related to the calcium channel were also highly upregulated.

Conclusion: The significant upregulation of cytochrome c gene expression and cytosolic calcium ion channel-related gene expression after H_2O_2 treatment suggests that ciliate mortality occurred through apoptosis. The formation of the cyst-like stage is considered a temporary form during the process of apoptosis. Information on the gene expression profile of *M. avidus* in response to H_2O_2 is expected to contribute to the understanding of the mechanism of action of therapeutic agents against this pathogen.

Keywords: Apoptosis; Hydrogen peroxide; *Miamiensis avidus*; Transcriptome analysis

Introduction

Miamiensis avidus is a highly pathogenic fish parasite with a wide range of aquatic hosts and is one of the most severe threats to many cultured marine fish, especially the olive flounder (*Paralichthys olivaceus*), as well as marine fish [1]. Recently, devastating outbreaks of *M. avidus* infection were reported in marine teleost fish along with wild elasmo-

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branchs [2].

It is well known that external stimuli or host immune responses lead to programmed cell death or cyst formation in various species of parasitic protozoa. The harsh conditions like drugs treatment trigger a path of programmed cell death in case of non-cyst-forming protists [3]. Hydrogen peroxide (H_2O_2) has been shown to play a key role in stress and programmed cell death in previous studies [4].

H_2O_2 and formalin are the most effective and widely applied chemicals for the treatment of scuticociliates [5] to date. H_2O_2 has been applied in many non-medical and medical fields owing to its stability in water and strong oxidizing properties. In particular, H_2O_2 has drawn attention as a suitable biocide in aquaculture, where its decomposition into non-toxic by-products is crucial. Because of these advantages, H_2O_2 has been approved by the U.S. Food and Drug Administration as a therapeutic agent for several fish pathogens, with applications including in freshwater cultured fish eggs, fingerlings, and against bacterial gill disease in adults [6]. Crosbie and Munday [7] found that *Uronema nigricans* was susceptible to H_2O_2 and was lysed completely at 250 ppm (the lowest concentration tested) for 60 minutes. The results in *Uronema marinum* incubated with H_2O_2 was similar to that of *U. nigricans*. When *U. marinum* was treated with a high concentration of H_2O_2, it was killed instantly, but it was not completely destroyed at lower concentrations (100 ppm) [8]. These reports indicate that H_2O_2 can be a potent therapeutic agent against pathogenic ciliates at moderate concentrations.

However, studies on transcriptional level and gene expression changes after H_2O_2 treatment are still insufficient, despite the excellent killing ability of H_2O_2. Therefore, we carried out an mRNA transcriptome analysis to compare the expressed gene profiles between the ciliate and cyst-like stages of *M. avidus* after H_2O_2 treatment. The comparison of the gene profiles between treated and non-treated groups will provide valuable information for understanding *M. avidus* destruction by H_2O_2.

Methods

1. *M. avidus* culture and cyst-like stage induction

The *M. avidus* used in this study was obtained from Pukyong National University (Busan, Korea), and was identified as *M. avidus* using species-specific oligonucleotide primers [9]. For the subculture, *M. avidus* was inoculated into a culture medium of 2% peptone, 1% yeast extract 0.5% sodium chloride, 10% fetal bovine serum, and 1% penicillin-streptomycin for 3–5 days at 22°C.

Treatment with 0.3% H_2O_2 in the scutica growth medium (15 mL) with 10% fetal bovine serum induced the cyst-like stage of *M. avidus* from the ciliated form. Cyst-like structures were confirmed by typical characteristics such as the loss of mobility, formation of an outer membrane, vacuole formation in the cytoplasm, and the loss of cilia. The cyst-like stage of *M. avidus* was harvested 72 hours after H_2O_2 treatment.

2. Library preparation and next generation sequencing

The total RNA was extracted using the Maxwell RSC simplyRNA Cells Kit (Promega, Madison, WI, USA) according to the manufacturer's protocols. The RNA concentration and RNA integrity were measured using NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA) and RNA Nano 6000 Assay Kit on an Agilent Bioanalyzer 2100 System (Agilent Technologies, Santa Clara, CA, USA), respectively. To obtain high-throughput transcript data of *M. avidus*, cDNA fragments were purified using the TruSeq Stranded Total RNA Sample Prep Kit (Illumina, San Diego, CA, USA) to select fragments of 100–150 bp. Sequencing was performed using an Illumina HiSeq4000 (Opentrons, Opentrons, NY, USA).

3. Sequence analysis and identification

The sequencing data were converted to raw reads by discarding adapters and low-quality reads and obtaining clean reads. Transcriptome assembly was performed based on the merged and clean paired-end reads generated by Illumina Hiseq4000 (Opentrons) using Trinity software. For the identification of functional transcripts, unigenes were searched against the National Center for Biotechnology Information non-redundant database using the BLASTx program with a cutoff *E*-value less than 1×10^{-5}.

4. Differentially expressed gene analysis

Clean reads were mapped onto the assembled transcripts. The read counts for each gene were obtained from the mapping results and normalized to the number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM). The differentially expressed genes (DEGs) were determined based on a difference of at least 2-fold in

the FPKM values and a p-value <0.05.

5. Gene ontology enrichment of DEGs

The gene ontology analysis was performed using the sequence annotation tool Blast2GO. After comparing similarities using the default parameters, three main categories (biological processes, cellular components, and molecular functions) were obtained.

Results

1. Sequence analysis and assembly of the transcriptome

Two cDNA libraries (ciliated and cyst-like) were sequenced on an Illumina HiSeq 2500 platform. A total of 98,172,410 and 60,346,680 raw reads were generated from the ciliated (control group) and cyst-like (induced group) databases, respectively (Table 1). After removing low-quality reads, 95,425,100 and 58,045,632 clean reads were obtained for each group, which mapped 97.2% and 96.2%, respectively. The transcripts were assembled into 29,699 single genes with an average length of 374 bp.

2. Gene functional annotation

The presumptive annotation of these transcripts was performed using BLASTx. The putative functions of 9,075 sequences (30.6%) of 29,699 unigenes were identified (Table 2).

3. Most abundantly expressed genes

The gene expression levels were estimated by calculating the abundance of reads in the transcriptome. The 20 most highly expressed transcripts are listed in Table 2. Protein *TAR1* was the most abundant gene in the two groups. However, most of the abundantly expressed genes were ribosomal protein genes that are essential for biological metabolism, such as 40S ribosomal protein S14, 60S ribosomal protein L8, Ubiquitin-60S ribosomal protein L40, and 60S acidic ribosomal protein P2. Among these 20 highly expressed genes, only elongation factor 1-alpha and peptidyl-prolyl cis-trans isomerase 1 were co-expressed in both stages (Table 3).

4. Stage-specific gene expression in the ciliated form and cyst-like stage of *M. avidus*

There were 5,967 DEGs among the 9,075 transcripts identified, and 50 of these DEGs were significantly different (p<0.05). Among these, 21 DEGs were upregulated and 29 were downregulated in H_2O_2 treated group (Tables 4, 5). The most significantly upregulated genes during the change to the cyst-like stage were caricain and cytochrome c oxidase genes. Cytochrome c oxidases are key enzymes in aerobic metabolism and closely related to oxidative stress. Genes related to the calcium channel, calcium transporter, gelsolin, and eIF2 were also highly upregulated. The expression of muscle contraction and cell motility-related genes, such as myosin-6 and myoglobin, were largely upregulated after induction into the cyst-like stage. In addition, the expression of the collagen alpha-1(III) chain and collagen alpha-2(I) chain genes, which encode the major components of collagen that strengthen and support many tissues of the body, also increased. However, the expression of metabolism-related genes, such as glyceraldehyde 3-phosphate dehydrogenase and pyruvate carboxylase 1, decreased after the induction of the cyst-like stage.

Table 1. Quality parameters of transcriptome sequencing of *Miamiensis avidus*

Stage	Raw reads	Raw bases	Raw bases (>Q30)	Clean reads	Clean base pairs	Low-quality reads
Ciliate	98,172,410	9,915,413,410	9,420,795,592	95,425,100 (97.2)	9,599,291,653 (96.8)	2,172,456 (2.2)
Cyst-like	60,346,680	6,095,014,680	5,724,436,494	58,045,632 (96.2)	5,844,404,085 (95.9)	1,794,418 (3.0)

Values are presented as number only or number (%).

Table 2. Gene functional annotation

Stage	Gene				Gene (>FPKM 1.0)			
	Expressed	Known	Novel	Unexpressed	Expressed	Known	Novel	Unexpressed
Ciliate	24,784	8,188	16,596	4,915	22,444	7,159	15,285	4,189
Cyst-like	24,003	6,444	17,559	5,696	23,237	6,339	16,898	3,396

FPKM, fragments per kilobase of transcript sequence per million base pairs sequenced.

Table 3. The top 20 most abundant genes in the ciliate and cyst-like stages

No.	Ciliate		Cyst-like	
	Name	Description	Name	Description
1	TAR1	Protein TAR1	TAR1	Protein TAR1
2	-	-	-	-
3	-	-	-	-
4	-	-	TBA_TETTH	Tubulin alpha chain
5	TU20	Polyubiquitin	RPL8	60S ribosomal protein L8
6	EF-1-alpha	Elongation factor 1-alpha	RLA2_EUPRA	60S acidic ribosomal protein P2
7	RPS15	40S ribosomal protein S15	ubl-1	Ubiquitin-like protein 1-40S ribosomal protein S27a
8	Rps14	40S ribosomal protein S14	GB1	Guanine nucleotide-binding protein subunit beta-like protein
9	-	-	TBA_TETTH	Tubulin alpha chain
10	RPS27AA	Ubiquitin-40S ribosomal protein S27a-1	EF-1-alpha	Elongation factor 1-alpha
11	RPL40	Ubiquitin-60S ribosomal protein L40	-	-
12	RPL8	60S ribosomal protein L8	RPL8	60S ribosomal protein L8
13	RPL35A	60S ribosomal protein L35a	RPL18	60S ribosomal protein L18
14	rps28b	40S ribosomal protein S28	Rps14	40S ribosomal protein S14
15	RPL17	60S ribosomal protein L17	-	-
16	-	-	HHT2	Histone H3
17	RLA2_EUPRA	60S acidic ribosomal protein P2	RPL40	Ubiquitin-60S ribosomal protein L40
18	RpS15Aa	40S ribosomal protein S15a	cyn-1	Peptidyl-prolyl cis-trans isomerase 1
19	RPL21	60S ribosomal protein L21	RPS10A	40S ribosomal protein S10-A
20	cyn-1	Peptidyl-prolyl cis-trans isomerase 1	rps19e	30S ribosomal protein S19e

Table 4. The top 21 most upregulated genes in the cyst-like stage

No.	Name	Description	Log_2 FC[a]	*p*-value
1	PPIII	Caricain	19.0	0.001
2	Mtco1	Cytochrome c oxidase subunit 1	16.3	0.003
3	mt-Co3	Cytochrome c oxidase subunit 3	15.2	0.006
4	Mtco2	Cytochrome c oxidase subunit 2	13.9	0.011
5	Myh6	Myosin-6	13.3	0.015
6	Mtnd1	NADH-ubiquinone oxidoreductase chain 1	13.0	0.018
7	Col3a1	Collagen alpha-1(III) chain	12.5	0.023
8	Gsn	Gelsolin	12.2	0.027
9	Mtnd2	NADH-ubiquinone oxidoreductase chain 2	11.8	0.033
10	Mb	Myoglobin	11.7	0.034
11	Col1a2	Collagen alpha-2(I) chain	11.4	0.041
12	Mtnd5	NADH-ubiquinone oxidoreductase chain 5	11.4	0.041
13	Myl2	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform	11.4	0.041
14	Tpm1	Tropomyosin alpha-1 chain	11.4	0.040
15	Apoe	Apolipoprotein E	11.2	0.046
16	Hba	Hemoglobin subunit alpha	11.2	0.044
17	Atp2a2	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	11.1	0.047
18	Eef2	Elongation factor 2	11.1	0.048
19	w	Protein white	8.9	0.036
20	Mtnd4	NADH-ubiquinone oxidoreductase chain 4	8.7	0.039
21	alh-9	Putative aldehyde dehydrogenase family 7 member A1 homolog	8.1	0.027

[a]log_2 FC, log_2 value of fold changes.

Table 5. The top 29 most downregulated genes in the cyst-like stage

No.	Name	Description	Log_2 FC[a]	*p*-value
1	GAPCP2	Glyceraldehyde 3-phosphate dehydrogenase GAPCP2, chloroplastic	−13.00	0.004
2	pyc-1	Pyruvate carboxylase 1	−9.84	0.020
3	RCA	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic	−9.82	0.020
4	LHCB5	Chlorophyll a-b binding protein CP26, chloroplastic	−9.71	0.021
5	RBCS F1	Ribulose bisphosphate carboxylase small chain F1, chloroplastic	−9.69	0.021
6	fech	Ferrochelatase, mitochondrial	−9.45	0.024
7	CAB36	Chlorophyll a-b binding protein 36, chloroplastic	−9.32	0.025
8	CAB8	Chlorophyll a-b binding protein 8, chloroplastic	−9.17	0.028
9	LHCP	Chlorophyll a-b binding of LHCII type 1 protein	−9.12	0.028
10	TPRP-F1	36.4 kDa proline-rich protein	−9.06	0.029
11	CAT3	Catalase-3	−9.05	0.029
12	PER42	Peroxidase 42	−8.92	0.031
13	CB12_PETHY	Chlorophyll a-b binding protein, chloroplastic	−8.91	0.031
14	THI1	Thiamine thiazole synthase, chloroplastic	−8.82	0.033
15	LHCA1	Chlorophyll a-b binding protein 6, chloroplastic	−8.80	0.033
16	AOX	Ubiquinol oxidase, mitochondrial	−8.66	0.020
17	Kdm4d	Lysine-specific demethylase 4D	−8.60	0.037
18	PSBR	Photosystem II 10 kDa polypeptide, chloroplastic	−8.52	0.038
19	CAB13	Chlorophyll a-b binding protein 13, chloroplastic	−8.47	0.039
20	LHCA4	Chlorophyll a-b binding protein 4, chloroplastic	−8.34	0.042
21	PSAL	Photosystem I reaction center subunit XI, chloroplastic	−8.30	0.043
22	psaD1	Photosystem I reaction center subunit II-1, chloroplastic	−8.30	0.043
23	Q949Q5_ARATH	-	−8.20	0.045
24	AGT1	Serine--glyoxylate aminotransferase	−8.19	0.045
25	PSBQ2	Oxygen-evolving enhancer protein 3-2, chloroplastic	−8.18	0.045
26	FD2	Ferredoxin-2, chloroplastic	−8.07	0.048
27	MT23_BRAJU	Metallothionein-like protein type 2, MT2-22	−8.06	0.048
28	RBCS	Ribulose bisphosphate carboxylase small chain, chloroplastic	−7.71	0.034
29	LHCB1.3	Chlorophyll a-b binding protein 1, chloroplastic	−7.70	0.033

[a]log_2 FC, log_2 value of fold changes.

5. Gene ontology enrichment analysis of the DEGs

Gene ontology enrichment is generally used to describe the biological roles of genes and their products. All DEGs were mapped to terms in the gene ontology database and compared to the whole transcriptome background to determine the function of the DEGs. The DEGs were classified into three major functional categories: biological processes, cellular components, and molecular functions. In the biological processes category, the DEGs were classified into 22 subcategories and the major DEGs were assigned into "metabolic process," "cellular process," and "single-organism process." Within the cellular component category, the DEGs were classified into 21 subcategories and many DEGs were classified as "cell," "cell part," "organelle," "organelle part," and "macromolecular complex." The molecular function categories included 23 subcategories and the subcategories of "binding" and "catalytic activity" were mostly related (Fig. 1).

Discussion

Here, we present comprehensive data on the transcriptome of the *M. avidus* cilia and the cyst-like stages after H_2O_2 treatment using the Illumina RNA-seq platform. One of the survival strategies for single-cell eukaryotes is to transform into a cyst stage in response to unfavorable environmental conditions. This phenomenon is commonly observed in pathogenic and free-living eukaryotes. When we treated *M. avidus* with H_2O_2 (0.3%), its shape gradually changed

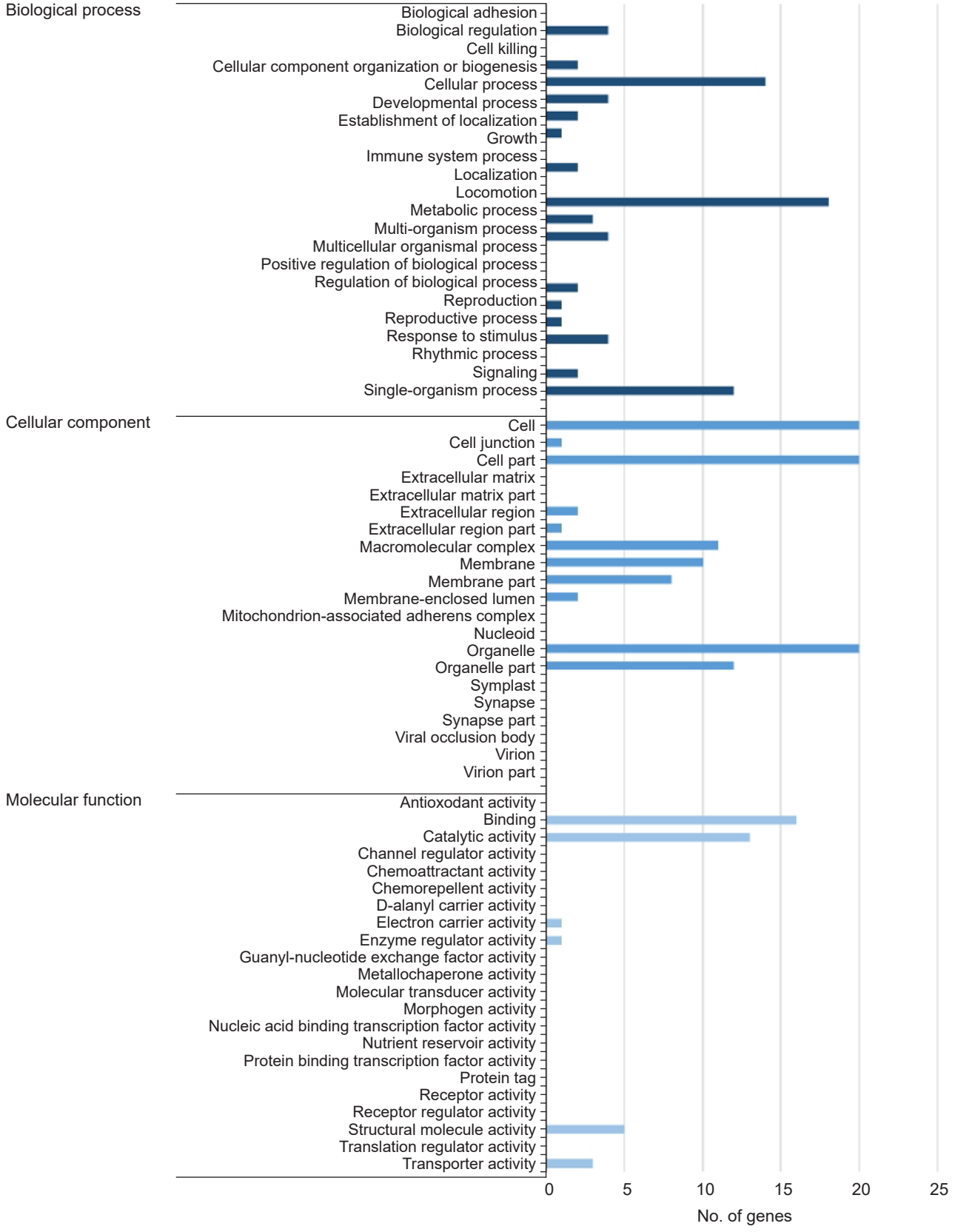

Fig. 1. Gene ontology (GO) annotation of differentially expressed genes (DEGs). The GO results are summarized into three main GO categories: biological processes, cellular components, and molecular functions.

and became a cyst-like round form. This overall structural change was accompanied by several other changes, such as the loss of cilia, vacuole formation, formation of an outer membrane, and the loss of active motion. Because of these changes, we hypothesized that the H_2O_2 treatment might induce a process similar to cyst formation in *M. avidus*. The induced cyst-like stage was converted to a small-sized ciliated form when it was returned to the normal media; however, it failed to divide further and eventually died. The life cycle of *M. avidus* is characterized by three stages: tomite, microstome, and macrostome. Although the tomite stage is non-feeding, it is a fast-swimming stage [10]. Therefore, the cyst-like stage induced by the H_2O_2 treatment was different from the normal cyst stage. We assumed that the cyst-like stage was an intermediate form during the death process.

Cyst formation is reportedly controlled by intracellular signal transduction pathways that convert environmental signals into gene expression changes [11,12]. Recent studies suggest that the cyclic adenosine monophosphate (cAMP) signaling pathway is probably a common mechanism of cyst formation in protists [13,14]. However, we could not find genes related to the cAMP signaling pathway, and the characteristic responses following encystment, such as autophagy-related gene expression, were not observed. Instead, we identified the caricain gene, which is well known as a class of papain-like cysteine proteases that are central to the immune defense system against invading pathogens or abiotic stress [15,16].

Previous studies have demonstrated that oxidative stress, such as H_2O_2 treatment, leads to apoptosis-like cell death in single-celled eukaryotes [17,18]. The morphological changes caused by oxidative stress lead to DNA fragmentation, increased vacuolization, nuclear condensation, and cell rounding in single-celled eukaryotes. We also observed some of these changes, such as vacuolization, cell rounding, and immobility.

Oxidative stress caused by elevated reactive oxygen species, such as H_2O_2, is known to cause programmed cell death through a process similar to apoptosis. Mitochondrial damage by these stimuli leads to excessive cytochrome c release, which can result in DNA damage and apoptosis [19,20]. We also found a significant increase in cytochrome c gene expression, which could explain the high rate of *M. avidus* death. When *Entamoeba histolytica* trophozoites were treated with H_2O_2, apoptosis-like death was induced, and one of the downstream effects was a cytosolic Ca^{2+} increase [21]. We also found upregulated gene expression related to calcium ion influx, such as the gelsolin and elongation factor 2 genes. Ribosomal protein S60 was highly expressed in both the ciliate and cyst stage. It is known to be involved in the regulation of translational initiation and protein synthesis in response to extracellular stimuli [22]. These findings suggest that the formation of the cyst-like stage of *M. avidus* after H_2O_2 treatment followed a similar process of encystment. However, the process was not a natural encystment process, but an intermediate form of cell death. The gene profile expressed during cell death suggests that this process is part of apoptosis.

The most markedly downregulated genes were those involved in glycolysis or gluconeogenesis, namely glyceraldehyde 3-phosphate dehydrogenase and pyruvate carboxylase 1. This was interpreted as a result of metabolic reduction. However, the gene expression levels of catalase and peroxidase were also significantly downregulated. These two genes are well-known enzymes whose optimal substrate is H_2O_2. It is presumed that the normal response corresponding to apoptosis was not accomplished due to prolonged exposure to H_2O_2 at a specific concentration. It can also be considered a high mortality effect of H_2O_2 on *M. avidus*.

Another point to note from our results was that the expression of chlorophyll-related genes significantly decreased. Most ciliates are heterotrophs, although they can be free-living or symbiotic depending on the food environment. *M. avidus* is a free-living species in marine environments when it is not parasitic on the fish host. However, chloroplasts have not been reported in ciliate groups, including *M. avidus*. Therefore, we speculate that the expression of genes with high homology to chloroplast-related genes was the result of the lack of reference genomes that were taxonomically close to *M. avidus*.

Taken together, the *M. avidus* cyst-like stage induced by H_2O_2 showed the upregulation of many apoptosis-related genes. These findings suggest that H_2O_2 treatment induces apoptosis-like cell death. However, the downregulation of two genes related to H_2O_2 breakdown suggests further study is required. In addition, a number of chloroplast-related genes were downregulated in the induced cyst-like stage. These results revealed a lack of scutica-related gene information. The comparison of the gene profiles between H_2O_2-treated and non-treated stages will provide valuable information for

understanding *M. avidus* destruction by H_2O_2.

Article information

Conflicts of interest

Hee-Jae Cha is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

Conceptualization: HK. Data curation: ARL. Formal analysis: HK. Funding acquisition: HJC, MSO. Methodology: HK, ARL, KYJ. Project administration: MSO. Visualization: EJK, MSO. Writing - original draft: ARL, MSO. Writing - review & editing: EJK, HJC, MSO. Approval of final manuscript: all authors.

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Validation of the association of the cystic duct fibrosis score with surgical difficulty in laparoscopic cholecystectomy

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Background: The level of surgical difficulty in laparoscopic cholecystectomy might be predictable based on preoperative imaging and intraoperative findings indicative of cholecystitis severity. Several scales for laparoscopic cholecystectomy have been developed, but most are complex, unverified, and not widely adopted. This study evaluated the association of the cystic duct fibrosis score (range, 0–3) with surgical difficulty in laparoscopic cholecystectomy.

Methods: Between July 2018 and November 2018, 163 laparoscopic cholecystectomy cases were retrospectively reviewed at a single center. Patients' demographics, preoperative laboratory data, operation time, complications, hospital stay, and cholecystitis severity grade were investigated. We also evaluated the associations of the Tokyo Guidelines 2018 and the Parkland grading scale with the cystic fibrosis score.

Results: The cystic duct fibrosis score was associated with preoperative white blood cells (p<0.001), preoperative platelet count (p=0.046), preoperative total bilirubin (p<0.004), preoperative C-reactive protein (p<0.001), operation time (p<0.001), cystic duct ligation time (p=0.002), estimated blood loss (p<0.001), postoperative complication (p=0.004), open conversion (p<0.001), and common bile duct injury (p=0.010). The cystic duct fibrosis score was also correlated with the Tokyo Guidelines 2018 and the Parkland grading scale (p<0.001). The cystic duct ligation time predicted the cystic duct fibrosis score and the Parkland grading scale, but not the Tokyo Guidelines 2018.

Conclusion: As a simple indicator of cholecystitis severity, the cystic duct fibrosis score can predict the surgical difficulty and outcomes of laparoscopic cholecystectomy.

Keywords: Cholecystitis; Gallbladder; Grade; Laparoscopic cholecystectomy

Introduction

Laparoscopic cholecystectomy (LC) is the gold-standard treatment of benign gallbladder (GB) disease, and one of the most common laparoscopic surgery [1,2]. However, inflammation of the GB is known to make surgery difficult, and increase postoperative complications [3,4]. There are several studies on the degree of GB inflammation, difficulty of surgery, and prediction of postoperative complications and progress in patients with LC [5-7]. Among many studies, Tokyo Guidelines 2018 (TG 18) and Parkland grading scale (PGS) are representative methods based on factors including preoperative symptoms, imaging, blood test, and intraoperative findings for the severity of acute cholecystitis (AC) [8-10].

However, sometimes the degree of cholecystitis based on

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preoperative images, symptoms, and blood tests are quite different from actual difficulties in surgery [11]. The most important part of LC is establishing a critical view of safety (CVS), cystic duct isolation, and ligation [12]. We assume that the degree of inflammation of the cystic duct will affect the technical difficulties of LC. We scored the hardness of the cystic duct as a cystic duct fibrosis score (CFS). This study aimed to validate the CFS as a predictor of surgical difficulty in LC and compare CFS with TG 18 and PGS.

Methods

Ethical statements: The protocol was approved by the Kosin University Gospel Hospital Institutional Review Board (KUGH 2021-10-019). The informed consent was waived because this design is a retrospective study.

1. Study population

Between July 2018 and November 2018, 165 cases of LC were retrospectively reviewed in a single tertiary medical center. We analyzed demographics, preoperative laboratory data, operation time, complications according to Clavien-Dindo classification, hospital stay, and severity grade of AC. In addition, we graded TG 18, PGS, and CFS, and compared preoperative, intraoperative, and postoperative parameters according to CFS grade. Moreover, we evaluated the association among TG 18, PSG, and CFS.

2. Surgical procedure

LC was performed by four hepato-biliary surgeons. The patients who had previously undergone open upper abdominal surgery or underwent single port LC were excluded. The GB is retracted over the liver with cephalic traction. The dissection begins with an incision of the peritoneum on both sides of GB to open up the hepatocystic triangle. After establishing CVS, the cystic duct and the cystic artery were isolated, ligated, and cut. Retrograde dissection of the GB from the liver bed is performed. If it was difficult to accurately identify the CVS, the fundus first approach was performed.

3. Definition

TG 18 diagnostic criteria were used for disease severity in patients with AC [13]. The grading system is as follows: I (mild AC), II (moderate AC), and III (severe AC). Information regarding patient medical history, physical examination, ultrasound findings, and laboratory values (white blood cell count, international normalized ratio, and complete blood cell count), as well as vasopressor use or mechanical ventilator status, were abstracted [14]. If there was no AC, we graded it as 0.

The appearance of GB was graded by the surgeon's description as PGS intraoperatively [10]: 1, normal appearing GB ("robin's egg blue"); 2, minor adhesions at the neck, otherwise normal GB; 3, hyperemia, pericholecystic fluid, adhesions to the body, distended GB; 4, adhesions obscuring the majority of GB or grade I-III with abnormal liver anatomy, intrahepatic GB, or impacted stone (Mirrizi syndrome); or 5, perforation, necrosis, inability to visualize the GB due to adhesions [10].

CFS was classified and scored by surgeon's description when dissecting a cystic duct (Fig. 1): 0, normal or no inflammation; 1, edematous but not hard; 2, not easy to dissect the cystic duct due to partial hardness (less than 50%); or 3, hard to dissect the cystic duct due to whole hardness (more than 50%).

Cystic duct ligation time was minutes from the start of surgery to the cystic duct ligation with clip or stapler. Clavien-Dindo classification II or more was defined as postoperative complications.

4. Statistics

Data included demographics, preoperative laboratory data, operation time, complications according to Clavien-Dindo classification, hospital stay, and severity grade of AC. The chi-square test was used to compare categorical variables. One-way analysis of variance test for independent samples was used to compare continuous variables. Continuous variables are presented as means with standard deviations. Linear by linear association was used to compare categorical variables among the CFS grade. Univariate linear regression was used for the analysis of the relation between cystic duct ligation time and operation time among severity grades of cholecystitis. In all analyses, a p-value of <0.05 was regarded as statistically significant. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for statistical analysis in the present study.

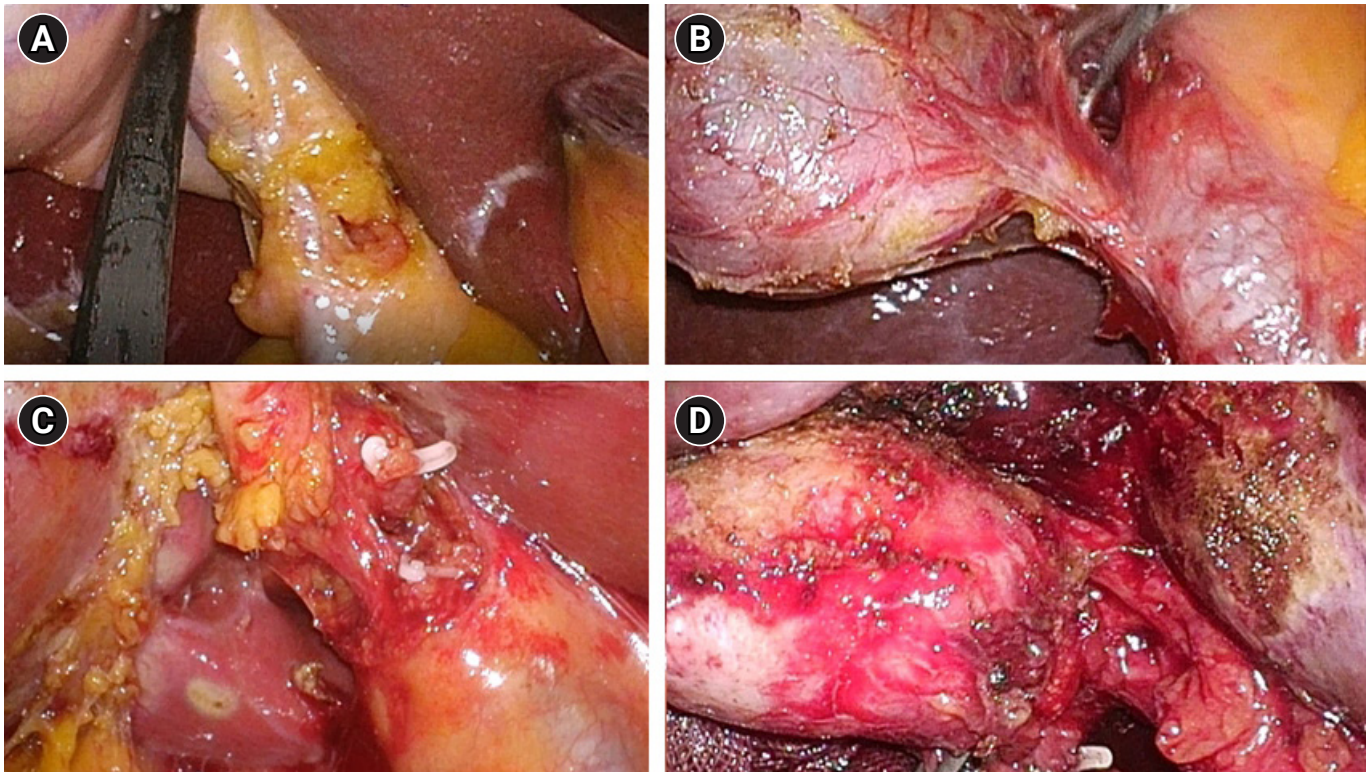

Fig. 1. Cystic duct fibrosis score. (A) Normal, (B) edematous, (C) partial hardness (<50%), (D) whole hardness (>50%).

Results

1. Demographics

A total of 85 males and 78 females were included in this study, their mean age was 56.6 years. the mean body mass index was 25.0 kg/m^2, the mean preoperative white blood cell (WBC) was 7,850 μL, the mean preoperative C-reactive protein (CRP) was 2.742 mg/dL. Eighty-one percent of them have gallstones and GB wall thickening was shown on computed tomography in 45.4% of them. In 31.9% of them needed preoperative percutaneous GB drainage. The mean operative time was 62.1 minutes and the hospital stay was 5.4 days. There were five cases of open conversion common bile duct (CBD) injury had happened in two cases during a laparoscopic procedure, we converted open surgery and inserted T-tube in CBD. The other case was difficult to proceed LC due to severe inflammation of GB. Postoperative complications included wound seroma, infection, and diarrhea (Clavien-Dindo II), one case of bile leak from CBD (Clavien-Dindo III) (Table 1).

2. Comparison of characteristics according to CFS

We compared the patient's characteristics according to CFS (Table 2). The higher CFS was associated with older patients (p=0.005), longer operative time (p<0.001) and cystic duct ligation time (p=0.002). Additionally, higher preoperative WBC (p<0.001) and CRP (p<0.001), lower preoperative platelet count (p=0.046), more estimated blood loss (p<0.001), high American Society of Anesthesiologists score (p=0.001), more GB stones (p=0.029), GB wall thickening (p<0.001), preoperative intervention (p<0.001), open conversion (p<0.001), CBD injury (p=0.010), and postoperative complications (p=0.004) in the higher CFS statistical significantly.

3. Associations of operative time and cystic duct ligation time with severity grade of cholecystitis

We analyzed the association among severity grades of AC with TG 18, PGS, and CFS. CFS is correlated well with TG 18, PGS (p<0.001) (Table 3). All three systems are well predicted the overall operation time of LC (p<0.001) (Table 4). In addition, it was analyzed that the cystic duct ligation time

Table 1. Demographics of patients who underwent laparoscopic cholecystectomy

Variable	Value (n=163)
Age (yr)	56.6±13.3
Sex (male/female)	85 (52.1)/78 (47.9)
BMI (kg/m^2)	25.0±4.0
Preoperative WBC	7,850±3,507
Preoperative CRP (mg/dL)	2.74±6.43
TG 18 (no/I/II/III)	97/58/7/1
PGS (1/2/3/4/5)	70/42/21/10/20
CFS (0/1/2/3)	81/40/13/29
Gallbladder stone	132 (81.0)
Gallbladder wall thickening	74 (45.4)
Previous PTGBD or ERBD	52 (31.9)
Operation time (min)	62.1±34.6
Estimated blood loss (mL)	17.1±79.4
Hospital stay (day)	5.4±3.2
Open conversion	5 (3.1)
CBD injury	2 (1.2)
Postoperative complications	14 (8.6)

Values are presented as mean±standard deviation, number (%), or number only.
BMI, body mass index; WBC, white blood cell; CRP, C-reactive protein; TG 18, Tokyo Guidelines 2018; PGS, Parkland grading scale; CFS, cystic duct fibrosis score; PTGBD, percutaneous transhepatic gallbladder drainage; ERBD, endoscopic retrograde biliary drainage; CBD, common bile duct.

was well predicted in CFS and PGS except TG 18.

Discussion

This study validated the CFS during LC and compared it with TG 18 and PGS CFS is a good predictor of the severity of AC through a comparison of preoperative laboratory data according to CFS; preoperative WBC (p<0.001), preoperative platelet (p=0.046), preoperative total bilirubin (p<0.004), and preoperative CRP (p<0.001). Through this, we can assume that CFS will be high during surgery if the preoperative inflammation-related laboratory data is severe.

In addition, CFS is a good predictor of the difficulty of LC, postoperative outcomes; operative time (p<0.001), cystic duct ligation time (p=0.002), estimated blood loss (p<0.001), postoperative complication (p=0.004), open conversion (p<0.001), and CBD injury (p=0.010). Therefore, if the CFS is high during surgery, there is a high possibility of complications during or after surgery, so be careful. In addition, we expected that the operation time and hospital stay might be longer. Moreover, it was possible to confirm the predictive ability of CFS once more through that CFS matches TG 18

Table 2. Comparison of characteristics according to the cystic duct fibrosis score

Characteristics	Score 0 (n=81)	Score 1 (n=40)	Score 2 (n=13)	Score 3 (n=29)	p-value
Age (yr), median	54.1	57.5	53.2	63.9	0.005
BMI (kg/m^2), median	24.5	25.2	25.7	25.9	0.359
Operation time (min), median	47.2	60.3	63.4	105.3	<0.001
Cystic duct ligation time (min), median	28.4	26.2	30.0	58.6	0.002
Preoperative WBC (μL), median	6,900	7,600	7,630	10,950	<0.001
Preoperative platelet (×10^3/μL), median	236	227	193	208	0.046
Preoperative TB (mg/dL), median	0.77	0.93	0.91	1.34	0.007
Preoperative CRP (mg/dL), median	0.81	3.09	1.35	7.94	<0.001
Estimated blood loss (mL), median	0	2.5	15.3	86.2	<0.001
ASA class (I/II/III/IV)	34/41/6/0	9/25/6/0	4/7/2/0	4/18/7/0	<0.001
TG 18 (no/I/II/III)	72/8/1/0	18/19/2/1	5/7/1/0	2/24/3/0	<0.001
PGS (1/2/3/4/5)	59/17/5/0/0	11/19/7/0/3	0/4/5/2/2	0/2/4/8/15	<0.001
GB stone (no/yes)	23/58	3/37	2/11	3/26	0.029
GB wall thickening (no/yes)	65/16	15/25	2/11	4/25	<0.001
Preoperative intervention (no/PTGBD/ERBD/both)	73/3/5/0	21/11/6/2	7/3/2/1	10/14/2/3	<0.001
Complications (≥C-D II, %)	3	4	0	7	0.004
Open conversion	0	0	0	5	<0.001
CBD injury	0	0	0	2	0.010

Score 0, normal or no inflammation; score 1, edematous but not hard; score 2, not easy to dissect the cystic duct due to partial hardness (less than 50%); score 3, hard to dissect the cystic duct due to whole hardness (more than 50%).
BMI, body mass index; WBC, white blood cell; TB, total bilirubin; CRP, C-reactive protein; ASA, American Society of Anesthesiologists; TG 18, Tokyo Guidelines 2018; PGS, Parkland grading scale; GB, gallbladder; PTGBD, percutaneous transhepatic gallbladder drainage; ERBD, endoscopic retrograde biliary drainage; C-D, Clavien-Dindo classification; CBD, common bile duct.

Table 3. Associations among the cholecystitis severity grades

	p-value		
	Tokyo Guideline 2018	Parkland grading scale	Cystic fibrosis score
Tokyo Guideline 2018	-	0.001	0.001
Parkland grading scale	0.001	-	0.001
Cystic fibrosis score	0.001	0.001	-

Table 4. Associations of operation time and cystic duct ligation time with the cholecystitis severity grades by univariate linear regression

	Cystic duct ligation time (min)		Operation time (min)	
	β±SE	*p*-value	β±SE	*p*-value
Tokyo Guideline 2018	7.217±4.899	0.148	22.616±4.155	<0.001
Parkland grading scale	7.318±2.168	<0.001	15.026±1.601	<0.001
Cystic fibrosis score	8.474±2.625	0.002	17.796±1.952	<0.001

SE, standard error.

(p<0.001) and PGS (p<0.001).

There are many grading methods to measure the severity of AC. These methods allow us to anticipate difficulties in surgery and predict postoperative results and complications and help us predict possible postoperative courses [7,8,10,14]. TG 18 is well known diagnostic criterion for the management of AC [15]. TG 18 is based on the preoperative symptom, blood test, radiologic findings, and presence of organ failure [16]. However, there is a limitation to predicting the difficulty of surgery with a TG 18 grade itself [14,17]. Moreover, the difficulty scoring of TG 18 listed 25 items that have 0 to 6 points [12]. The complemented scoring system is considered too complex to be applied clinically. In contrast to TG 18, Sugrue et al. [7] established a new scoring system, using operative findings such as GB appearance, distension, adhesion, or impacted stone of the GB during surgery. This is the first introduced classification of AC with intraoperative findings. However, These scoring systems are also complex, with multiple inputs and grades, limiting the practicality of using these scores during operation.

The American Association for the Surgery of Trauma (AAST) developed a grading system for AC as I to V. Individualized anatomically driven injury patterns were used in AAST grading for assessment of AC [14]. AAST grade was validated and reported to be superior to TG 18 in the prediction of postoperative mortality, complications, and the need for preoperative GB drainage [14]. The weakness of AAST grade is no significant differences in clinical events were noted between AAST grades 1 and 2 cholecystitis [18].

Meanwhile, PGS is simple, intuitive, and easy reproducible among surgeons when compared to a previous grading system based on intraoperative findings [10]. PGS is classified as the degree of inflammation of GB with the level of adhesion and covering of omentum on GB [10]. PGS was demonstrated that higher grades have a longer operative time, increased operative difficulty, and increased postoperative complication rate compared to lower grades [11]. However, even though grade 5, after peeling off omentum covering GB, the operation would be easier due to less inflammation than expected in some cases. Securing CVS is one of the most important processes in LC. The difficulty of this process is directly linked to postoperative complications including bile duct injury.

We evaluated cystic duct fibrosis because the harder the cystic duct, the more difficult to secure CVS [2,5]. Then it results in prolonged surgery, bailout procedure, unexpected bleeding, and possible bile duct, vessel, or other organ injuries [12]. TG 18 mentioned a fibrotic change in the Calot's triangle area as one of the difficulty scores for intraoperative findings [12]. But we grade cystic duct fibrosis and hypothesized CFS itself is related to surgical difficulty and postoperative outcomes. This study shows CFS is related to the severity of cholecystitis and the surgical difficulty of LC. Of course, it is difficult to detect the degree of AC and predict the difficulty of surgery by CFS alone. If too many details are included, it becomes a complex system and becomes difficult to apply in practice [19,20].

We acknowledge this study has several limitations. First, the proportion of patients of cholecystectomy was relatively small from a single institution. Second, the cystic duct is

hidden by the peritoneum or covered with omentum in AC. Therefore, before peeling off adhesion tissue or incision of the peritoneum, accurate evaluation of cystic duct fibrosis is limited. Third, CFS has aspects of subjective assessment depending on the surgeon. In addition, surgical skills and policies of treatment may vary depending on the surgeon. Nevertheless, CFS is significantly correlated well with the severity of AC, operation time, cystic duct ligation time, hospital stay, and complications. In addition, CFS is well-matched with TG 18 and PGS. CFS could be used as a simple predictor of severity of AC and difficulty of LC and postoperative outcomes.

Article information

Conflicts of interest

Young Il Choi is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Conceptualization: HHM, YIC, DHS. Data curation: HHM, YIC. Methodology: HHM. Writing- original draft: HHM, JHJ, YIC. Writing- review & editing: JHJ, YIC, DHS. Approval of final manuscript: all authors.

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Hepatic steatosis changes after early gastric cancer surgery

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Background: Nonalcoholic fatty liver disease dramatically improves after bariatric surgery, primarily due to improvements in hepatic insulin sensitivity. Since the procedure for gastric cancer surgery is very similar to that for bariatric surgery, we investigated changes in fatty liver following gastrectomy for gastric cancer according to the type of surgery.

Methods: We evaluated hepatic steatosis in 212 early gastric cancer patients using Hounsfield units (HUs) on non-contrast computed tomography preoperatively and 6, 12, and 24 months after surgery. We compared the preoperative and postoperative liver-to-spleen HU ratio according to the type of surgery: Billroth I, Billroth II, and total gastrectomy with Roux-en-Y reconstruction.

Results: The initial results (liver/spleen HUs and the liver-to-spleen HU ratio) did not significantly differ according to surgical group. After surgery, only patients who underwent total gastrectomy with Roux-en-Y exhibited significant changes in the liver-to-spleen HU ratio at 6 months. In 26 patients who had higher initial HU levels of the spleen than the liver, the liver-to-spleen HU ratio significantly increased from 0.836 to 1.115 at 6 months, 1.109 at 12 months, and 1.102 at 24 months (p<0.01).

Conclusion: Significant changes in hepatic steatosis were found in even normal patients (with higher liver than spleen HU values) who underwent total gastrectomy with Roux-en-Y. Patients who initially had fatty liver also showed a significant increase in the liver-to-spleen HU ratio. These results suggest that total gastrectomy with Roux-en-Y reconstruction can have a positive effect on the improvement of hepatic steatosis.

Keywords: Hepatic steatosis; Liver disease; Stomach neoplasm

Introduction

Many studies have suggested that bariatric surgery has a remarkable effect on clinical manifestations of type 2 diabetes (T2DM). Buchwald et al. [1] reported that 82% of patients in their meta-analysis achieved resolution of clinical and laboratory manifestations of T2DM in the first 2 years after bariatric surgery, while 62% remained free of T2DM for more than 2 years after bariatric surgery. Furthermore, the rates of T2DM resolution differed by surgery type [1,2]. A meta-analysis showed T2DM resolution rates according to surgical type: 48% in laparoscopic adjustable gastric banding, 84% in Roux-en Y gastric bypass (RYGB), and 98% in biliopancreatic diversion [2].

In addition to T2DM, nonalcoholic fatty liver disease (NAFLD) also improved after bariatric surgery [3,4]. The spectrum of NAFLD ranges from simple hepatic steatosis to more severe nonalcoholic steatohepatitis, fibrosis, end-stage liver disease, and hepatocellular carcinoma [5]. The prevalence of NAFLD is between 10% and 25% in the

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general Korean population [6]. NAFLD in Korea is closely associated with several components of metabolic syndrome including obesity, hypertension, T2DM, and dyslipidemia, as also seen in Western countries [6]. Insulin resistance and hyperinsulinemia might play roles in the pathogenesis of NAFLD in patients with obesity and of normal body weight [4,6]. The mechanism underlying improvement of NAFLD after bariatric surgery suggests early improvement of hepatic insulin sensitivity [4,7].

According to the Korea National Cancer Incidence Database from 1999 to 2012, stomach cancer was one of the leading primary cancers [8]. In particular, stomach cancer is predicted to be the most common cancer in males. The age-standard mortality rate per 100,000 cases of stomach cancer was 7.8 in Korea in 2015 [8]. The treatment of choice for stomach cancer is surgery, with a procedure very similar to that of bariatric surgery. As a result, we compared changes in fatty liver following gastric cancer surgery based on which surgery.

Methods

Ethical statements: This study was approved by the Institutional Review Board of Kosin University Gospel Hospital (KUGH 2016-12-001). The informed consent was waived because this design is a retrospective study.

1. Study design and participants

A retrospective review was conducted with the medical records of 374 patients who underwent gastric cancer surgery in Kosin University Gospel Hospital from 1 January to 31 December of 2013. Among the 374 patients, 212 had early gastric cancer.

We only enrolled early gastric cancer patients who underwent computed tomography (CT) scan before surgery and 6, 12, and 24 months after surgery. We evaluated hepatic steatosis using Hounsfield units (HUs) on non-contrast CT images. Hepatic attenuation only and liver-to-spleen (LS) ratio in HUs were calculated prior to the operation and at 6, 12, and 24 months after surgery. We compared the preoperative and postoperative results according to reconstruction method: Billroth I, Billroth II, and Roux-en Y.

2. Measurements

Body weights and heights were measured using standardized techniques and equipment with participants wearing light indoor clothing without shoes. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Blood samples were obtained after at least 8 hours of fasting. CT scans were performed with SOMATOM Sensation or SOMATOM Definition Flash (Siemens, Munich, Germany).

3. CT assessment of hepatic steatosis

An endocrinologist who was educated by a radiologist measured hepatic and splenic CT attenuation on non-enhanced CT scans. Fat accumulation of the liver decreases the attenuation of CT [9]. On three cross-sections taken at different hepatic levels, the hepatic CT attenuation was assessed by selecting three circular regions of interest with an area of at least 2 cm^2. By excluding areas of hepatic vascular and biliary structures, the largest possible region was chosen. Hepatic CT attenuation was calculated as the mean of the three measurements. Splenic CT attenuation was calculated through the same method.

The presence of fatty liver disease was defined as CT evidence of hepatic steatosis. The LS ratio was used to normalize hepatic attenuation with splenic attenuation [9]. CT steatosis was defined as a LS ratio less than one, and hepatic attenuation divided by splenic CT attenuation less than one, which has previously been proven to correlate with the presence of steatosis in liver biopsy and to be particularly specific for the presence of steatosis [10].

4. Statistical analysis

Differences in baseline characteristics were evaluated by one-way analysis of variance in the three surgical groups. Furthermore, paired *t*-tests were used to compare the changes of CT steatosis with follow-up in each surgery group. All data are presented as means with standard deviations. A p-value <0.05 was considered statistically significant. All analyses were performed using SPSS, version 23.0 (IBM Corp., Armonk, NY, USA).

Results

This study was composed of a total of 212 early gastric cancer patients with a mean age of 58.43±10.53 years. Among them, 62.3% (n=132) underwent surgery with Billroth I,

22.2% (n=47) with Billroth II, and 15.5% (n=33) with total gastrectomy with Roux-en Y bypass surgery.

Preoperative baseline characteristics were not significantly different among surgical groups except for weight and BMI (Table 1). HUs of liver, HUs of spleen, and LS ratio were not significantly different among the groups.

After gastric cancer surgery, all groups exhibited a reduction in BMI (Table 2). In total gastrectomy in the Roux-en Y group, total cholesterol, low-density lipoprotein, and triglyceride levels significantly decreased 6 months after surgery. High-density lipoprotein cholesterol increased but was not statistically significant (Table 2).

Fig. 1 shows the changes in LS ratio with hepatic steatosis according to type of surgery. No significant changes were shown after surgery in the Billroth I or Billroth II operation group. However, there was significant increase in the LS ratio in the Roux-en Y group from 1.140 to 1.233 at 6 months, 1.223 at 12 months, and 1.261 at 24 months after surgery compared to the preoperation values (p=0.03).

In this study, most participants exhibited no hepatic steatosis before surgery. We selected 26 patients who had LS ratio <1, indicating the presence of hepatic steatosis [10], before surgery. Fig. 2 shows the changes in LS ratio after surgery. The LS ratio increased from 0.836 to 1.115 at 6 months, 1.109 at 12 months, and 1.102 at 24 months after surgery (p<0.001). Their initial mean BMI was 25.88±3.61 kg/m^2, which is not considered obesity [11,12]. The mean reduction in BMI after gastric cancer surgery was 4.26±1.26 kg/m^2 (Table 3).

Discussion

Fatty liver disease is a predisposing condition of T2DM and increases insulin resistance [13]. Meta-analysis revealed that the improvement or resolution rate of hepatic steatosis after bariatric surgery was close to 90% [3]. The subjects in the included studies were severely obese, with BMIs ranging from 43.9 to 56 kg/m^2 before bariatric surgery, classifying them as obesity class III according to the World Health Organization (WHO) [11,12]. After surgery, the BMIs remarkably decreased as a percentage reduction of mean BMI, ranging from 19.11% to 41.76%. These results pertaining to

Table 1. Baseline characteristics of the study participants according to the type of surgery

Characteristics	Billroth I	Billroth II	Total gastrectomy with Roux-en-Y	*p*-value
No. of participants	132 (62.3)	47 (22.2)	33 (15.5)	
Sex, male/female	79/53	34/12	27/7	
Age (yr)	58.28±10.09	57.83±10.79	60.15±12.09	0.051
Weight (kg)	62.46±10.05	69.16±9.08	65.17±11.75	0.002
Height (cm)	163.90±8.67	167.35±8.45	165.31±6.98	0.037
BMI (kg/m^2)	23.21±2.98	24.74±3.35	23.83±3.88	0.046
Fasting glucose (mg/dL)	101.52±26.72	104.13±23.20	94.32±10.60	0.744
HbA1c (%)	7.33±1.82	7.05±1.11	6.18±0.64	0.378
Total cholesterol (mg/dL)	176.12±28.92	179.06±36.09	176.97±26.62	0.886
HDL (mg/dL)	47.86±10.30	46.93±14.25	44.76±10.91	0.507
LDL (mg/dL)	104.08±30.28	97.53±38.03	108.29±29.29	0.180
Triglycerides (mg/dL)	119.64±28.55	129.19±67.75	129.56±59.44	0.498
GOT (IU/L)	24.36±8.55	22.98±8.31	25.50±9.33	0.598
GPT (IU/L)	23.22±9.90	27.09±15.61	24.09±10.15	0.082
ALP (IU/L)	71.99±19.41	66.00±19.95	69.74±23.43	0.167
γ-GTP (U/L)	36.17±43.70	32.05±30.15	40.50±42.22	0.770
Liver HUs	53.53±6.38	51.44±6.81	50.95±9.35	0.074
Spleen HUs	45.50±3.20	45.32±3.67	44.83±3.51	0.568
Liver-to-spleen ratio	1.180±0.144	1.140±0.178	1.140±0.165	0.257

Values are presented as number (%) or mean±standard deviation.
BMI, body mass index; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase; HUs, Hounsfield units.

Table 2. Serial changes of baseline characteristics after 6 months according to the surgery type

Characteristics	Billroth I			Billroth II			Total gastrectomy with Roux-en-Y		
	Preoperative	6 Months	*p*-value	Preoperative	6 Months	*p*-value	Preoperative	6 Months	*p*-value
Weight (kg)	62.46±10.05	57.34±9.05	0.017	69.16±9.08	56.00±1.41	0.144	65.17±11.75	60.08±11.62	0.033
BMI (kg/m^2)	23.21±2.98	21.56±2.67	0.022	24.74±3.35	21.88±3.76	0.106	23.83±3.88	20.19±2.89	0.036
Total cholesterol (mg/dL)	176.12±28.92	168.94±30.99	0.001	179.06±36.09	159.04±22.48	0.020	176.97±26.62	157.51±29.24	<0.001
HDL (mg/dL)	47.86±10.30	53.85±1.15	0.203	46.93±14.25	46.54±13.46	0.634	44.76±10.91	49.77±12.61	0.258
LDL (mg/dL)	104.08±30.28	96.39±32.13	0.005	97.53±38.03	84.96±26.48	0.065	108.29±29.29	91.39±21.55	0.030
Triglycerides (mg/dL)	119.64±28.55	116.37±57.64	0.708	129.19±67.75	117.92±58.41	0.137	129.56±59.44	108.04±39.74	0.048
GOT (IU/L)	24.36±8.55	26.43±10.11	0.046	22.98±8.31	27.21±11.81	0.078	25.50±9.33	27.42±7.44	0.249
GPT (IU/L)	23.22±9.90	22.46±11.54	0.539	27.09±15.61	28.65±21.42	0.922	24.09±10.15	25.00±9.90	0.502
ALP (IU/L)	71.99±19.41	79.01±18.92	<0.001	66.00±19.95	87.54±66.76	<0.001	69.74±23.43	77.22±18.22	0.125
r-GTP (U/L)	36.17±43.70	19.4±14.95	<0.001	32.05±30.15	69.73±257.53	0.225	40.50±42.22	22.21±18.97	0.549
Liver HUs	53.53±6.38	52.51±4.13	0.006	51.36±6.81	51.12±5.58	0.469	50.95±9.35	51.53±7.59	0.590
Spleen HUs	45.50±3.20	43.63±3.49	<0.001	45.4±3.67	44.95±3.56	0.397	44.83±3.51	42.13±3.59	0.011
Liver-to-spleen ratio	1.180±0.144	1.213±0.149	0.300	1.142±0.178	1.142±0.134	0.841	1.140±0.165	1.233±0.217	0.043

Values are presented as mean±standard deviation.
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase; HUs, Hounsfield units.

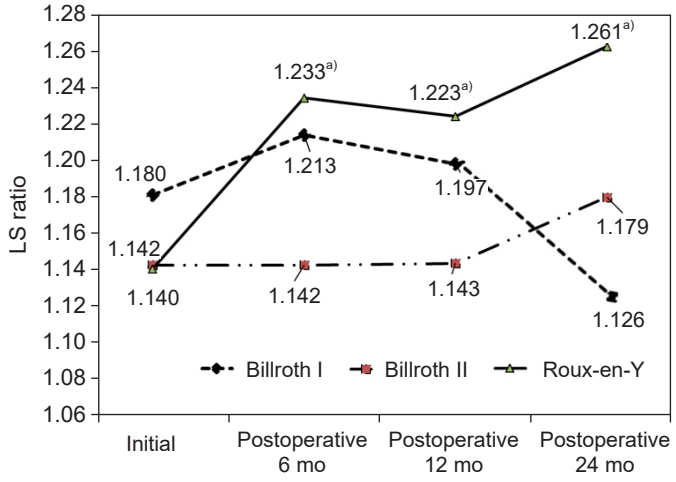

Fig. 1. Changes in the liver-to-spleen (LS) ratio according to type of surgery. [a]p<0.05 compared to the preoperative values.

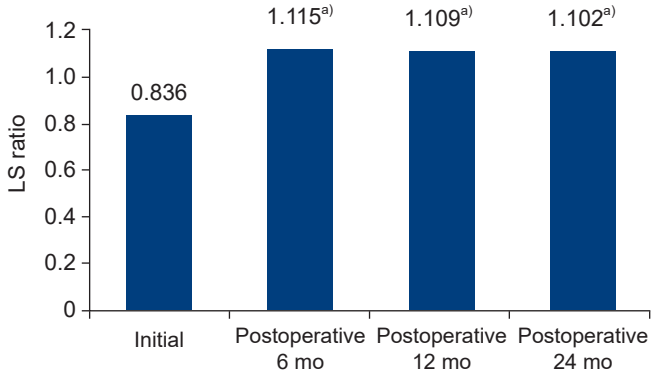

Fig. 2. Changes in the liver-to-spleen (LS) ratio among patients who had hepatic steatosis before surgery. [a]p<0.05 compared to the preoperative values.

resolution of hepatic steatosis after bariatric surgery might be influenced by weight reduction. Therefore, remission of T2DM after bariatric surgery could be partially explained by resolution of hepatic steatosis.

The methods for bariatric and stomach cancer surgeries are very similar, and there have been reports that improvements in T2DM after gastric cancer surgery are similar to those achieved with bariatric surgery. One retrospective study reported that T2DM was resolved in 15.1% and improved in 30.4% of patients after stomach cancer surgery [14]. Another study revealed a better effect with T2DM. In patients who underwent Billroth II surgery, 85% exhibited improvement, while 88% of patients who underwent Roux-en-Y showed improvement [15]. However, to the best of our knowledge, no study has addressed fatty liver changes after stomach cancer surgery.

In this study, only 26 patients had fatty liver (LS ratio <1) before surgery. Their LS ratio was dramatically improved 6 months after stomach cancer surgery, and the improvement was sustained for 24 months. Almost all participants

Table 3. Serial changes of baseline characteristics after surgery in 26 patients who had hepatic steatosis before surgery

Characteristics	Preoperative	6 Months	12 Months	24 Months	*p*-value
BMI (kg/m^2)	25.88±3.61	23.93±5.04	22.78±1.67	22.74±2.37	0.02
Total cholesterol (mg/dL)	187.05±29.57	155.79±31.15	160.54±38.34	159.84±36.67	0.03
HDL (mg/dL)	42.51±7.77	41.82±9.64	46.68±11.15	45.39±10.96	0.03
LDL (mg/dL)	112.45±42.80	90.71±22.71	92.27±33.89	88.81±27.87	0.33
Triglycerides (mg/dL)	175.87±74.63	160.14±72.10	128.27±75.40	148.83±97.67	0.54
GOT (IU/L)	24.68±8.14	25.77±8.82	32.70±38.24	28.04±6.79	0.20
GPT (IU/L)	28.81±10.57	24.10±18.77	34.27±41.50	24.50±9.17	0.04
ALP (IU/L)	68.39±24.35	80.57±23.44	80.70±23.85	77.77±22.97	0.11
γ-GTP (U/L)	36.22±27.76	21.05±12.36	25.62±20.82	23.72±22.24	0.01
Liver HUs	40.03±7.29	49.03±7.50	48.76±4.08	49.26±4.08	<0.01
Spleen HUs	46.39±2.98	44.47±3.47	44.33±4.02	44.89±3.76	0.03
Liver-to-spleen ratio	0.836±0.141	1.115±0.234	1.109±0.155	1.102±0.134	<0.01

Values are presented as mean±standard deviation.
BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; ALP, alkaline phosphatase; γ-GTP, gamma glutamyl transpeptidase; HUs, Hounsfield units.

had normal body weight before surgery. Their preoperative BMI was lower than that of patients with obesity, defined as BMI of 25 kg/m^2 or greater [11,12]. The participants did not achieve the hepatic steatosis criteria by CT LS ratio before gastric cancer surgery. Interestingly, hepatic CT attenuation and LS ratio increased after surgery even though they remained within the normal range. These results suggest that normal-weight patients and not only fatty liver patients can experience improvement after stomach cancer surgery. The reduction of mean BMI after surgery in this study was much smaller than in a previous meta-analysis [3]. These results suggest that improvement of hepatic steatosis after gastric surgery is due to another mechanism in addition to weight reduction. Our observations are consistent with those from previous studies. A number of studies has shown that improvement in the manifestations of metabolic syndrome including NAFLD after bariatric surgery is not solely dependent on weight [7,16-18]. Bojsen-Moller et al. [7] reported improvement in basal hepatic insulin sensitivity within 1 week after RYGB in obese T2DM patients, long before any significant weight reduction was achieved [19]. They suggested that this immediate improvement of hepatic insulin sensitivity could be a response to calorie restriction after bariatric surgery. They also showed an increment of glucagon-like peptide-1 (GLP-1) secretion that reached almost 5-fold peak concentration and a 12-fold area under the curve after oral glucose exposure with RYGB, which might be one mechanism for weight-independent improvement after surgery. The relationship between GLP-1 and hepatic steatosis has been addressed in several *in vivo* and *in vitro* studies [20-22]. These studies have suggested that GLP-1 acts directly on hepatocytes through activation of metabolic signaling pathways including the insulin signaling and AMP-activated protein kinase (AMPK) pathways. Therefore, improvement of insulin sensitivity is achieved. The AMPK pathway is associated with hepatic lipogenesis [21]. GLP-1 binds to its receptor and enhances the increase in cyclic AMP, a known activator of AMPK [23]. This process accelerates the phosphorylation of AMPK and reduces lipogenesis in hepatocytes [21]. This could explain the improvement of hepatic steatosis after gastric cancer surgery even though a much lower reduction of BMI was achieved compared to previous meta-analysis.

Previous studies have shown that bypassing the proximal gut for delivery of nutrients can enhance GLP-1 secretion and create differences in degree of improvement of fatty liver and T2DM after bariatric surgery according to surgery type [1,24,25]. RYGB or biliopancreatic diversion exhibited better results than sleeve gastrectomy [1]. Therefore, the importance of bypassing the proximal intestine has been demonstrated [24,25]. Our study results are consistent with other studies, as total gastrectomy with the Roux-en Y group exhibited significant changes in the LS ratio as well as the lipid profile compared to other surgery groups. This is a remarkable result, considering that the length of the bypass limb in the Roux-en-Y group (usually 30–40 cm) is very

short compared to that in bariatric surgery (usually from the duodenum to 50–100 cm distal to the ligament of Treitz) [26]. This finding indicates that bypassing the proximal intestine even by a very short length could improve metabolic disease. Recently, several studies have suggested that GLP-1 and bile acid are related to this response [27].

Due to the retrospective design of this study, data collection was limited. Liver biopsy to confirm the states of hepatic steatosis was not performed. There is a limitation in not being able to collect data on whether patients have underlying diseases such as T2DM, hypertension, hyperlipidemia, drinking alcohol, and taking drugs related to underlying diseases. These data could influence as confounding variables. In addition, there is no patient with distal gastrectomy with Roux-en Y anastomosis, so the difference according to the anastomosis method cannot be confirmed. In the Billroth II patient group, there was no change in the L to S ratio even though the weight loss was the greatest before and 6 months after surgery. In this study, it was not possible to determine whether alcohol consumption or an underlying medical condition had an effect.

Tests for insulin resistance, sensitivity, and GLP-1 level were not included. In addition, this study involved stomach cancer surgery, not bariatric surgery. Therefore, the participants were early gastric cancer patients with initial relatively normal body weight and normal hepatic steatosis state. Although these were the conditions, there were significant changes in hepatic steatosis after surgery.

Recently, the 5-year survival rates and remission rates were shown to be very high in early gastric cancer patients [28]. Therefore, surgical improvement of cancer remission and metabolic disease can affect quality of life and mortality rates in early gastric cancer patients with metabolic disease. In the case of gastrectomy patients, it can be inferred that metabolic diseases like obesity, NAFLD, and T2DM tend to improve.

Stomach cancer surgery results in significant improvements of hepatic steatosis in early gastric cancer patients with fatty liver. Especially, total gastrectomy with Roux-en Y can increase liver HUs and LS ratios even in normal patients who exhibited no fatty liver before surgery. These results can help clinicians determine the effectiveness of the treatment of metabolic disease in postoperative patients with early gastric cancer with metabolic disease.

Article information

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: BKK. Data curation: DL, JHJ. Formal analysis: DL, JHJ. Methodology: DL. Project administration: SK, YSC. Visualization: SO, BKK. Writing - original draft: SO, BKK. Writing - review & editing: KHK, YK, KWS, KY. Approval of final manuscript: all authors.

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New approach to learning medical procedures using a smartphone and the Moodle platform to facilitate assessments and written feedback

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Background: To overcome communication obstacles between medical students and trainers, we designed serial learning activities utilizing a smartphone and web-based instruction (WBI) on the Moodle platform to provide clear and retrievable trainer feedback to students on an objective structured clinical examination (OSCE) item.

Methods: We evaluated students' learning achievement and satisfaction with the new learning tool. A total of 80 fourth-year medical students participated. They installed the Moodle app (the WBI platform) on their smartphones and practiced an endotracheal suction procedure on a medical simulation mannequin while being evaluated by a trainer regarding competence in clinical skills on the smartphone app. Students' competency was evaluated by comparing the scores between the formative assessment and the summative assessment. The degree of satisfaction and usefulness for the smartphone and WBI system were analyzed.

Results: The means (standard deviations, SDs) of the formative and summative assessments were 8.80 (2.53) and 14.24 (1.97) out of a total of 17 points, respectively, reflecting a statistically significant difference ($p<0.05$). The degree of satisfaction and perceived usefulness of the smartphone app and WBI system were excellent, with means (SDs) of 4.60 (0.58), and 4.60 (0.65), respectively.

Conclusion: We believe that the learning process using a smartphone and the Moodle platform offers good guidance for OSCE skill development because trainers' written feedback is recorded online and is retrievable at all times, enabling students to build and maintain competency through frequent feedback review.

Keywords: Clinical skills; Feedback; Objective structured clinical examination; Web based instruction

Introduction

The processes of observation and procedural practice for in-patient and out-patient care are an important part of medical education [1,2]. These processes involve corrective feedback typically provided verbally, and the majority of learning is obtained via observation and verbal feedback [3]. In general, students with limited clinical experience do not easily achieve competence by observation and verbal feedback alone, particularly because such feedback is not always clear. To facilitate procedural skill training, trainer feedback needs to be retrievable and available regardless of

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time and place [4-6].

Lack of timely feedback from teacher to student is a critical communication obstacle to overcome in medical education [7]. Immediate feedback allows students to refine clinical skills as they are being practiced [8,9]. Currently, feedback is typically provided verbally, but not always timely, and without the student's continuous participation in the provided guidance, proficiency can be lost. Furthermore, memory can become distorted over time, which can alter a student's understanding of verbal feedback. Therefore, timely written feedback is necessary so students can retrieve it later for self-directed learning [4,10,11]. The medical education field needs a reliable and tangible tool to ensure clear written feedback is continuously utilized by students for preparation of their clinical skills.

Furthermore, due to the coronavirus disease 2019 (COVID-19) pandemic, in-person education has become limited. Thus, virtual education is accepted as a new normal form of education [12,13]. Nevertheless, some aspects of medical education, such as clinical skills, are difficult to implement virtually because they require direct feedback. In this context, we needed a teaching tool to minimize in-person verbal explanation to prevent participants from being infected with the COVID-19 virus via droplets of saliva. It was believed that a modular object-oriented dynamic learning environment (Moodle) platform and smartphone app would provide an educational environment for trainers to give written feedback to students in a timely manner.

Therefore, we designed serial learning activities within the smartphone app and the Moodle platform to guarantee clear and retrievable trainer feedback. We then evaluated students' learning achievements and their satisfaction with the new learning tool.

Methods

Ethical statements: The study was approved by the Institutional Review Board of the Kosin University Gospel Hospital (KUGH 2020-11-028). Informed written consent was exempted due to the retrospective nature of this study.

A total of 80 fourth-year medical students participated in this study. Students were asked to complete formative assessment, self-reflection, summative assessment, and a learning-tool survey (Fig. 1). All assignments were created using Moodle version 3.0 software (Martin Dougiamas, Perth, Australia; http://www.moodle.org/). Students were instructed to install the Moodle app, a web-based instruction (WBI) platform, on their smartphones (Fig. 2). They were asked to practice an "endotracheal suction" procedure (one of the objective structured clinical examination [OSCE] items used to measure a student's clinical competence) on a medical simulation mannequin while being evaluated in person by a trainer on the smartphone app. We used the results from the students' submitted assignments for this study.

1. Formative assessment

Students were asked to learn an endotracheal suction procedure with a provided manual and movie clips on an e-learning consortium website. After that, a formative assessment was performed to evaluate the current status of each student's skills wherein a trainer in charge of the students' learning provided feedback based on their performance. Before the procedure, students were required to log in to the Moodle app via their smartphone and open a quiz designed as the formative assessment. The students then handed their smartphones to the trainer, who was responsible for filling out the assessment. Students began the endotracheal suction procedure in front of the trainer. Since the quiz was locked with a pass code, the quiz questions were invisible. To begin the assessment, the trainer input a passcode on the landing page of the quiz and completed the formative assessment based on the student's demonstrated skillset.

The quiz consisted of 17 questions which dealt with critical check points for a proficient endotracheal suction procedure. Each of the questions had rated answers based on the level of student competence demonstrated. When a trainer selected one of the answers, corresponding feedback would automatically generate. However, the trainer could add additional constructive feedback if necessary. After completing the formative assessment, students would read both the algorithm-generated answers in addition to the trainer's on-spot written feedback.

2. Self-reflection

After completing the formative assessment, students could see the quiz questions and answers as well as feedback

Fig. 1. WBI website for formative assessment, self-reflection, summative assessment, and feedback. Students accessed the web-based instruction (WBI) website for online endotracheal suction practice (A), where they completed the formative assessment (B), self-reflection (C), summative assessment (D), and survey (E). OSCE, objective structured clinical examination.

online. The quiz results and feedback provided the students with guidelines regarding their competency in the endotracheal suction procedure. They reflected on their performance and the written feedback from the trainer and reported essays for self-reflection. We used the "assignment" activity within Moodle, on the WBI, for students to submit their self-reflection essay, in which they had the opportunity to consider their performance and correct their weaknesses.

3. Summative assessment

Three days after completing the formative assessment, the students performed another round of endotracheal suction to demonstrate their learned skillset. As in the formative assessment, students presented their smartphones right before the procedure, and the trainer assessed their performance using the Moodle app. The quiz questions on the summative assessment were the same as those on the formative assessment. The trainer compared students' performances with those recorded during the formative assessments via quizzes and checked whether their levels of competency had improved.

4. Survey

We used the "survey" activity in Moodle to obtain student feedback about satisfaction and usefulness of the educational system utilized during this study. The survey consisted of one subjective question and 14 objective questions. Of the 14 objective questions, half asked for degree of satisfaction and half about the usefulness of the educational system. Students were asked to specify their level of agreement to a statement on a 5-point Likert scale: 1, strongly disagree; 2, disagree; 3, neither agree nor disagree; 4, agree; 5, strongly agree [14]. The subjective question was intended to collect additional opinions that had not been considered in the objective portion of the survey.

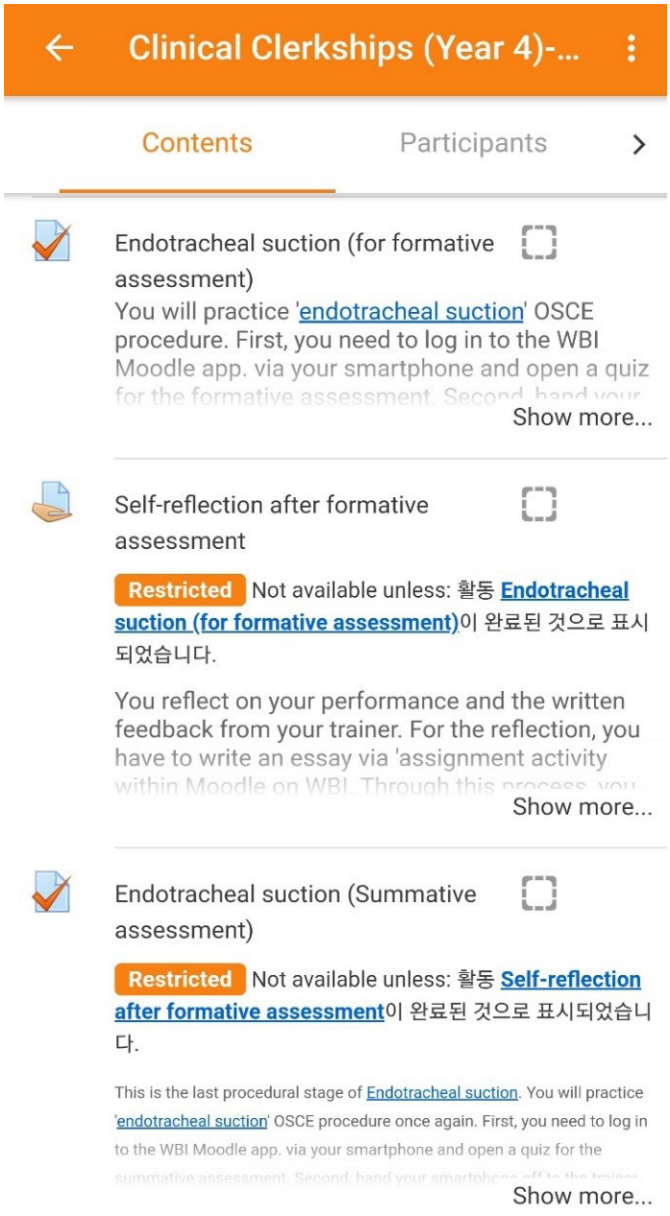

Fig. 2. Smartphone app screen for the endotracheal suction practice class.

5. Statistical analysis

Cronbach's α was measured to gauge the internal consistency of survey questions. Paired-samples *t*-test was used to analyze the difference of mean between the formative assessment and the summative assessment. Statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA). Statistical significance was established at *p*-value <0.05.

Results

1. Difference of students' learning achievements between the formative and summative assessments

We analyzed the difference between the scores of formative and summative assessments to confirm how much students' learning had improved. The formative and summative assessment questions were designed to check that the essential requirements had been met for performing a correct endotracheal suction procedure. Students are required to prove that they have enough knowledge and skill to safely and correctly complete the procedure without any adverse effects to the patient such as hypoxemia and pneumothorax. Many aspects have to be taken into consideration to begin the endotracheal suction procedure; depth of suction, saline instillation, pre-oxygenation, disconnection of a tube, size of suction catheter, suction ion, aseptic procedure, adverse effects, etc. The quiz questions were designed to cover all these concerns related to a safe and successful procedure. From this perspective, it was assumed that the score of formative and summative assessments represented the students' overall proficiency in the procedure.

The means (standard deviations, SDs) for the formative and summative assessments were 8.80 (2.53) and 14.24 (1.97) out of a total of 17 points, respectively (Table 1). There was a significant difference in score between the two assessments (p<0.001). Students' proficiencies have been remarkably improved (162.8% increase in mean score). Only

Table 1. Formative and summative assessment scores

	No.	Mean	Standard deviation	T score	*p*-value[a]
Formative assessment	80	8.80	2.53	−17.149	<0.001
Summative assessment	80	14.24	1.97		

A total of 80 participants completed the formative and summative assessments.
[a]The statistical significance of differences between the scores for the two assessments was evaluated with the paired-samples *t*-test, and the mean scores were significantly different.

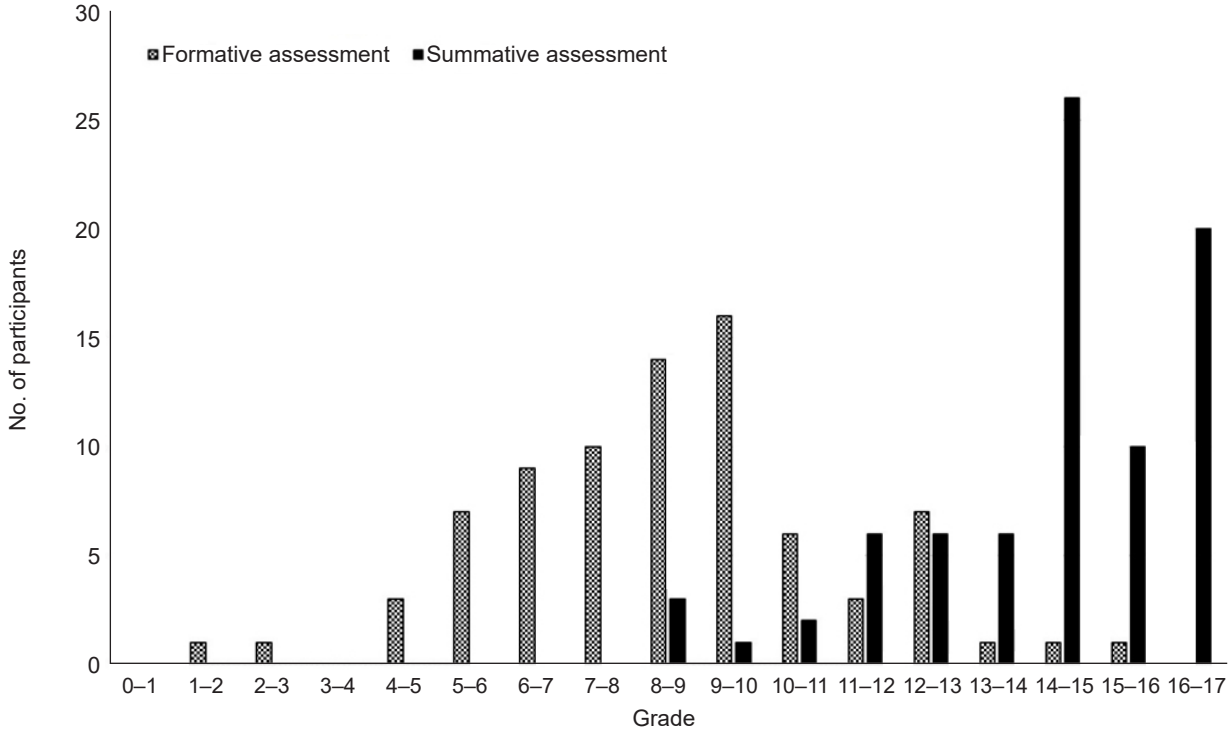

Fig. 3. Frequency distribution of student grades on the formative assessment and summative assessment.

two students scored more than 14 of 17 (2.5%) at formative assessment; this increased to 56 students (70%) at the summative assessment (Fig. 3).

2. Students' satisfaction with the new learning tool

A total of 56 students (70%) reported degree of satisfaction and usefulness of the smartphone app and WBI system (Table 2). The mean (SD) of the degree of satisfaction was 4.60 (0.58), and the mean (SD) for smartphone app and WBI system usefulness was 4.60 (0.65). The average internal consistency of the survey for degree of satisfaction and usefulness was greater than 0.9 (Cronbach's α=0.918 and 0.919, respectively).

Students were highly satisfied with the newly introduced written feedback and assessment system via a smartphone and WBI website (question no. 3, mean=4.72). The students indicated that the written feedback was helpful (question nos. 4 and 5, mean=4.80, respectively). Students were asked whether the feedback provided during practice was consistent with that provided in a real-world situation. The students responded positively to this question. However, the degree of satisfaction was relatively lower when compared to other questions (question no. 6, mean=4.30).

About the usefulness of a smartphone and WBI system, students felt it was easy to operate (question no. 8, mean=4.58), and that it helped them reflect on their performance and maintain competence (question nos. 9 and 11, mean=4.73 and mean=4.66, respectively). They indicated that the assessments and feedback could potentially be reused for their portfolio in the future (question no. 13, mean=4.41).

Discussion

Effective feedback on medical student performance from formative or summative assessments can be a cornerstone to improve learning outcomes [15-18]. We evaluated a practical and implementable method to provide retrievable and clear feedback to medical students. To accomplish this goal, a virtual learning environment was presented in Moodle [19,20]. Smartphone use in clinical and medical education has been reported to be effective for enhancing patient care and medical education [21-23]. Therefore, we combined these two tools, Moodle platform (WBI website) and smart-

Table 2. Degree of satisfaction and usefulness of the smartphone app and WBI system in an OSCE class

Questions	Min	Max	Mean	SD	Cronbach's α (n=64)
Degree of satisfaction					
1. I was pleased with the endotracheal suction practice.	2	5	4.50	0.73	0.913
2. I was confident after the endotracheal suction practice that I could perform endotracheal suction on a real patient.	3	5	4.44	0.73	0.922
3. The feedback and assessments via the smartphone app and WBI system during the OSCE class were helpful for practicing endotracheal suction.	3	5	4.72	0.49	0.917
4. The teacher pointed out my mistakes and gave me proper feedback.	4	5	4.80	0.41	0.920
5. I think the teacher was well prepared for class.	4	5	4.80	0.41	0.918
6. The feedback obtained from practice was consistent with that provided in treatment with real patients.	3	5	4.30	0.75	0.923
7. I am satisfied with this endotracheal practice class as a whole.	3	5	4.69	0.53	0.913
Usefulness of the smartphone and WBI system					
8. It was easy to operate the smartphone app and WBI system for endotracheal suction practice.	2	5	4.58	0.69	0.914
9. This system was helpful for reflection by providing feedback on needed corrections prior to the next assessment.	4	5	4.73	0.45	0.914
10. After formative assessment, utilizing the feedback and self-reflection activities in the app, I noted academic improvement between the two assessments.	3	5	4.66	0.54	0.913
11. I think the feedback from the smartphone app could help maintain my competence, even after completion of the OSCE class.	2	5	4.66	0.60	0.916
12. I could systematically learn endotracheal suction skills with the help of the smartphone app and WBI system.	3	5	4.58	0.59	0.914
13. The assessments and feedback for endotracheal suction practice were stored online and could be useful for my portfolio design.	1	5	4.41	0.87	0.921
14. Other OSCE practice classes could utilize the smartphone app and WBI system that were applied to this practice class.	1	5	4.30	0.85	0.926

One trainer gave feedback to all students during all classes in order to eliminate the possibility of bias caused by differences in teaching style. This written feedback was given using the smartphone app.
WBI, web-based instruction; OSCE, objective structured clinical examination; SD, standard deviation.

phone using several Moodle functions that could be implemented in both the WBI website and a smartphone app.

During formative assessment for this study, a trainer input comments as feedback on smartphones provided by students, allowing accurate and efficient feedback. We also embedded online comments that the trainer could use to answer each quiz item, which saved time and facilitated the assessment process [24]. With this approach, the students' primary responsibility during procedural practice class with the trainer was to concentrate on the procedure based on trainer feedback. We used the quiz as the basis for feedback because many checkpoints in the endotracheal suction procedure can be presented as questions, and various degrees of competency were evaluated through quiz responses. Also, extra feedback can be included as personalized comments. Trainers had the option to provide feedback by selecting pre-provided answers or writing comments.

After completing the formative assessment, students reflected on their activities based on the feedback on the smartphone app and were able to respond by strengthening their weak points [25]. Our goal was that students would correct their mistakes during reflection, on which they were asked to submit an essay which the trainer assessed for accuracy of the students' insights and provided additional online feedback.

Table 1 shows that students' mean procedural skills improved significantly from 8.80 (SD, 2.53) in formative assessment to 14.24 (SD, 1.97) in summative assessment (p<0.001). Translating feedback into action through self-reflection is a process for catalyzing positive behavioral change [21]. Timely and retrievable feedback should increase self-reflection by enabling immediate corrective behavior change.

Use of a smartphone and a WBI system could be perceived as inconvenient to both students and trainers compared to in person education. However, the system used in this study was described as easy and helpful by students

(Table 2). The degree of satisfaction reported by students was excellent (4.30–4.80), with excellent internal consistency (Cronbach's α >0.9). Trainer activities in this teaching environment are more intensive than in traditional teaching because the trainer is required to record feedback on the smartphone consistently and accurately while observing the students' performance. The automatically generated feedback helped alleviate the burden of this task.

The COVID-19 pandemic has had a significant effect on life, including society, culture, the economy, and education. It has accelerated online education, which is not common in medical training [26-28]. It is essential to guarantee quality education while maintaining social distancing during the COVID-19 pandemic [29,30]. To achieve this, student performance could be evaluated and critiqued in a hybrid way via offline practice and online communication. The method introduced herein, harnessing the Moodle online platform and a smartphone for the written feedback, can help achieve this two-pronged goal. Digital communication in medical education is gaining importance [31]. Interactive online medical activities can be a rich source of personal reflection and learning and can contribute to a student's portfolio to be used for future professional development [32,33]. Written feedback and activities using the WBI and smartphone can be included as useful items in the portfolio because they are stored online and are easily extracted.

We only tested the system developed in our institution on one OSCE item, limiting generalizability to the rest of the OSCE items. Further studies with a larger number of items for application of this educational system are necessary. The improvements in proficiency of the endotracheal suction procedure by students could be partly ascribed to well-written feedback or a well-organized learning environment supported by the Moodle platform and use of a smartphone. However, a detailed analysis was not performed to weigh the degree of contribution between them. As stated, the Moodle platform and smartphone app facilitating the written feedback and assessments were the main factors considered in this study. Other factors which could have potentially affected the results were not analyzed. Although the difference between the formative and summative assessments is significant, this cannot fully explain whether the improvement in procedural learning has been obtained by the combination of the written feedback and the Moodle platform or through the repeated assessment and learning.

We believe a learning process using a smartphone and the Moodle platform can offer good guidance for building OSCE skills because online written feedback is retrievable at all times, allowing students to increase/maintain competency via feedback review even after completion of the OSCE class. Because the teaching method used in this study has never been reported and because teaching and learning environments vary depending on the course subject, further studies that apply this newly designed teaching method are warranted. The educational system evaluated herein could be a good alternative for clinical procedural education during periods when social distancing is necessary, such as during the COVID-19 pandemic.

Article information

Conflicts of interest

Hyunyong Hwang is an editorial board member of the journal but was not involved in the peer reviewer selection, evaluation, or decision process of this article. No other potential conflicts of interest relevant to this article were reported.

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Author contributions

Conceptualization: HL, HH. Data curation: SSL, HL, HH. Formal analysis: HL, HH. Methodology: HL, HH. Visualization: SSL, HL, HH. Writing - original draft: SSL, HL. Writing - review & editing: HH. Approval of final manuscript: all authors.

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Case report

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Metastatic ameloblastoma with postoperative accelerated tumor growth treated with carboplatin and paclitaxel: a case report

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Ameloblastoma is a histologically benign odontogenic epithelial tumor that rarely metastasizes. However, metastasis to the lungs can occur, usually years after the development of the primary tumor. Here, we present the case of a 63-year-old woman with metastatic ameloblastoma in the lungs that developed 12 years after surgery for the primary lesion. As is typical for metastatic ameloblastomas, the tumor was incidentally found on radiography and surgically removed. However, the tumor exhibited accelerated progression with pleural metastasis after surgical removal, which is unusual in metastatic ameloblastoma. The patient was successfully treated with carboplatin/paclitaxel and showed a partial response to tumor progression, implying that this approach can be safely used in the absence of a standard treatment regimen.

Keywords: Ameloblastoma; Carboplatin; Neoplasm metastasis; Paclitaxel; Postoperative complication

Introduction

Ameloblastoma is the second most common odontogenic tumor that mainly involves the mandible and maxilla [1]. It is a benign tumor arising from the odontogenic epithelium [2], with slow invasive growth and a high recurrence rate (50%–72%). Although rare (<2%), metastasis is primarily distant [1]. Histopathologically, metastatic ameloblastoma does not differ from non-metastatic ameloblastoma, making it impossible to predict its clinical behavior [3]. The most common site of metastasis is the lungs (≥5%) [4]. The current treatment choice for primary ameloblastoma is surgery, including both curettage and radical surgery. However, the treatment for metastatic ameloblastoma is yet to be established [3,4].

We present a case of metastatic ameloblastoma which developed in the patient's lung 12 years after a primary lesion surgery. This case is unusual owing to the rapid tumor progression with pleural metastasis that was observed after the resection of metastatic ameloblastoma of the lung. Due to the absence of a regular treatment regimen, the patient was treated with chemotherapy using carboplatin and paclitaxel.

Case

Ethical statements: This study was approved by the Institutional Ethics Committee of Wonkwang University Hospital (WKUH 2020-06-015-001). Informed consent was obtained from the patient.

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In March 2020, a 63-year-old woman with pulmonary nodules was referred to the Wonkwang University Hospital, Iksan, Korea. She had a history of recurrent ameloblastoma of the right mandibular ramus, for which she underwent mass excision and bone grafting in 1983 and 2001. The pulmonary nodules were incidentally found on a regular chest radiography in 2013. A chest computed tomography (CT) scan revealed two lesions in the right lung, a 13 mm-sized heterogeneous enhancing solid nodule with peripheral ground-glass opacity (GGO) in the right lower lobe (RLL) (Fig. 1A), and an 8 mm-sized noncalcified nodule in the right middle lobe (RML) (Fig. 1B). The patient refused the recommended diagnosis and treatment. She returned asymptomatic in March 2020 with enlarged pulmonary nodules and agreed to undergo treatment.

The patient had a normal physical examination. On the CT scan, both the nodules had remarkably increased in size. The RLL mass was 45 mm in size, well-defined, lobulated, and heterogeneously enhanced with peripheral GGO; it abutted the right major fissure and diaphragm, causing pleural retraction (Fig. 2A). The RML mass was 14 mm in size, spiculated-margined, and solid enhancing, which also caused pleural retraction (Fig. 2B). Considering her past diagnosis, metastatic ameloblastoma was more likely than a primary lung cancer with a metastatic nodule. We performed resection of the anterior basal segment of the RLL and wedge resection of the RML. Postoperative histopathological evaluation confirmed the diagnosis (Fig. 3). No histological signs of malignancy were observed. Positron emission tomography with CT only showed signs of postoperative pulmonary inflammation. The patient was discharged without indication for adjuvant therapy.

In July 2020 she was admitted to the emergency room with mild dyspnea (mMRC 1 grade). Chest X-ray revealed a massive right pleural effusion. Analysis of the aspirated pleural fluid showed a lymph-dominant exudate and suspected malignant pleural effusion, but the cytological examination was negative for malignancy. After drainage of the pleural effusion, a chest CT was performed, which showed locoregional tumor recurrence at the surgical stump site with multiple pleural metastases in the right hemithorax (Fig. 4A). Subsequently, thoracoscopic pleural biopsy was performed. Pathology confirmed metastatic ameloblastoma. Chemotherapy is recommended for multiple pleural metastases and lesions that are not amenable to surgical resection or radiotherapy. Our multidisciplinary tumor

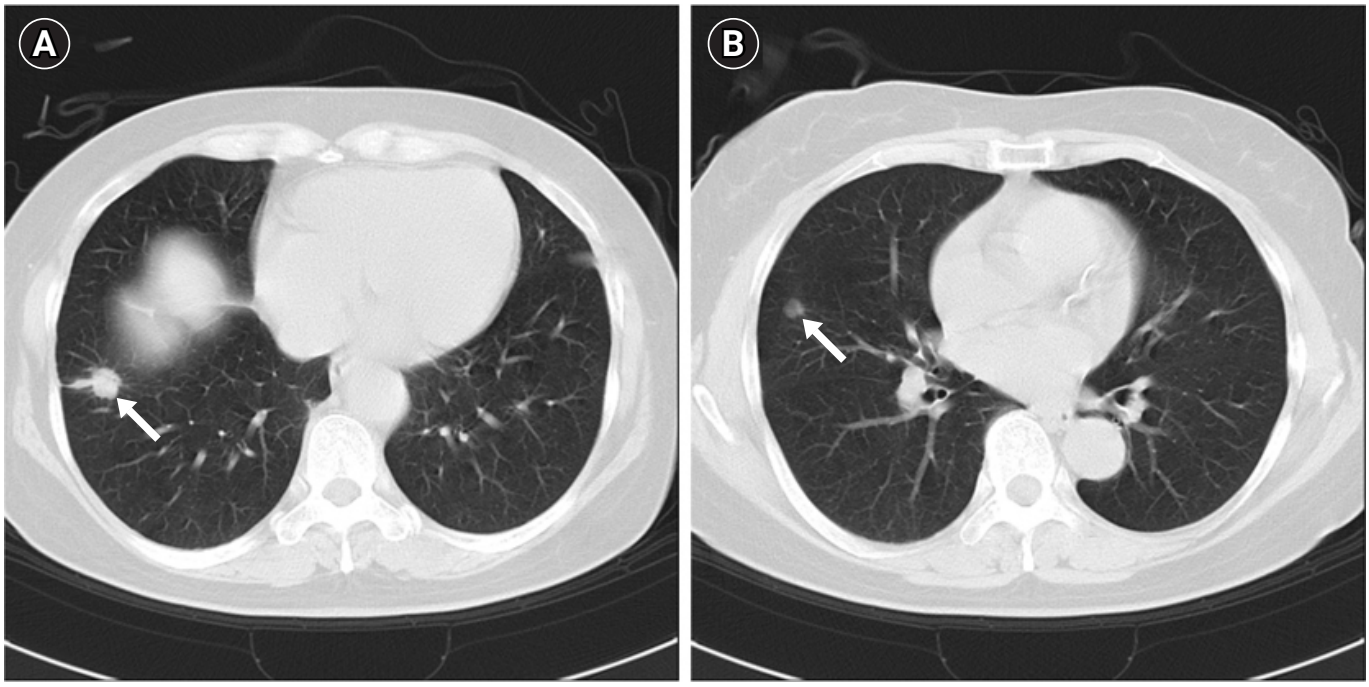

Fig. 1. Initial chest computed tomography performed in October 2013. (A) A 13-mm heterogeneous enhancing solid nodule with a peripheral ground-glass opacity in the right lower lobe (arrow). (B) An 8-mm small, noncalcified nodule in the right middle lobe (arrow).

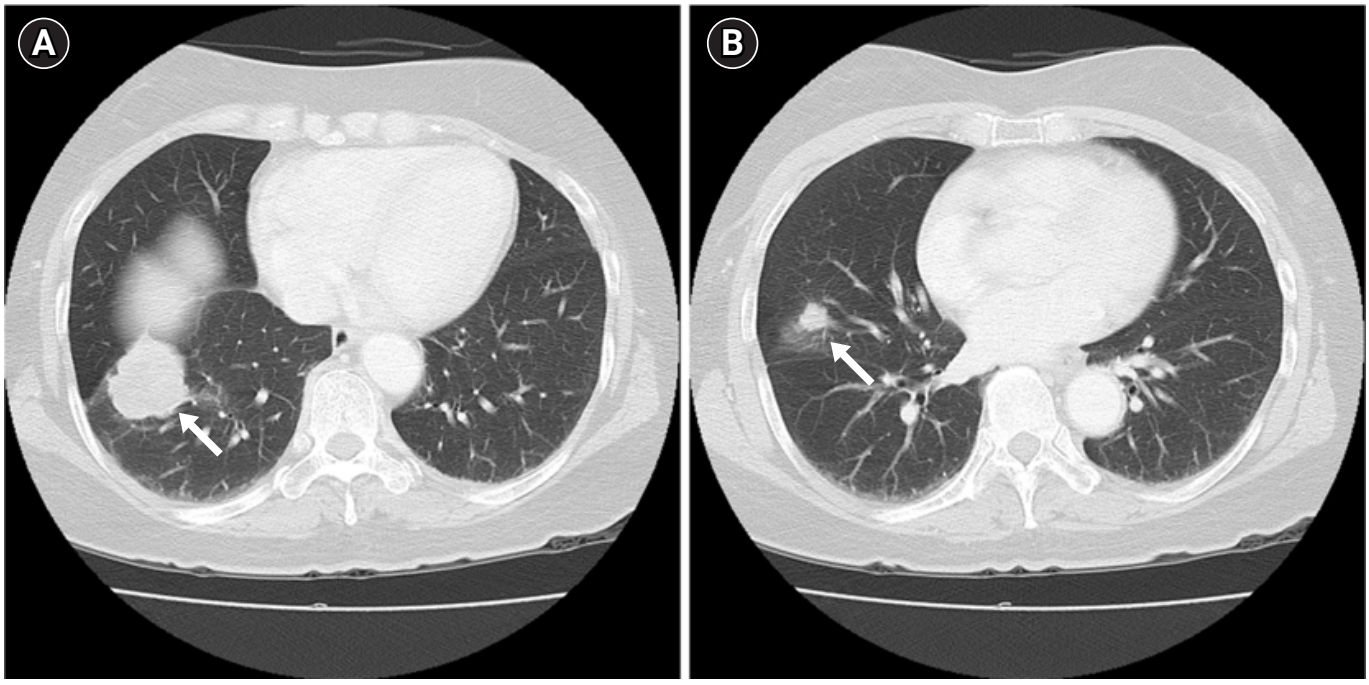

Fig. 2. Follow-up chest computed tomography performed in March 2020. (A) A 45-mm well-defined, lobulated, heterogeneously enhancing mass with a peripheral ground-glass opacity, abutting the right major fissure and diaphragm and causing pleural retraction in the right lower lobe (arrow). (B) A 14-mm, spiculated-margin, enhancing solid nodule with pleural retraction in the right middle lobe (arrow).

board decided to treat with combined chemotherapy of carboplatin and paclitaxel for six cycles which were administered intravenously every 21 days. After three cycles, CT scans were taken, which showed variable responses to the chemotherapy. Three additional cycles were administered. Patient developed grade 4 neutropenia during the last cycle but recovered. Post-treatment, follow-up CT scans demonstrated a radiological partial response (Fig 4B). A year later, the patient is clinically asymptomatic.

Discussion

Among odontogenic and maxillofacial bone tumors, ameloblastomas (11%) are the second most common neoplasm [5]. Despite its local invasiveness and frequent recurrence, ameloblastoma is benign, slow-growing, and asymptomatic. Primary ameloblastomas are usually diagnosed between the third and fifth decades of life without any race or gender predilections [3,6].

Despite its 50% to 72% rate of relapse, metastasis to distant organs or lymph nodes rarely occur [7]. Distinguishing metastatic ameloblastoma from ameloblastic carcinoma is important. In 2017, the World Health Organization classified metastatic ameloblastoma as a benign odontogenic tumor [8]. Primary and metastatic ameloblastomas are histopathologically identical to benign ameloblastomas. The histologic findings observed in the primary tumor are not specific for predicting metastasis [8]. The known risk factors of metastasis are the tumor duration, the extent of initial disease, multiple surgical procedures, and radiation therapy [4,9].

The average disease-free interval is 18 years (range, 3–45 years) [6]. In this case, it was 12 years. Biopsy is needed to confirm the diagnosis of malignant ameloblastoma. As in our case, it is often found incidentally by imaging studies in asymptomatic patients. Regular radiographic follow-ups of asymptomatic patients with a history of ameloblastoma are important for early detection.

When our patient returned in 2020, we observed that the metastatic ameloblastoma had grown relatively slow since 2013. Three months after the surgical removal, unusual rapid tumor progression with pleural metastasis was observed in the metastatic ameloblastoma. Even considering the possibility of recurrence at the surgical stump, this is

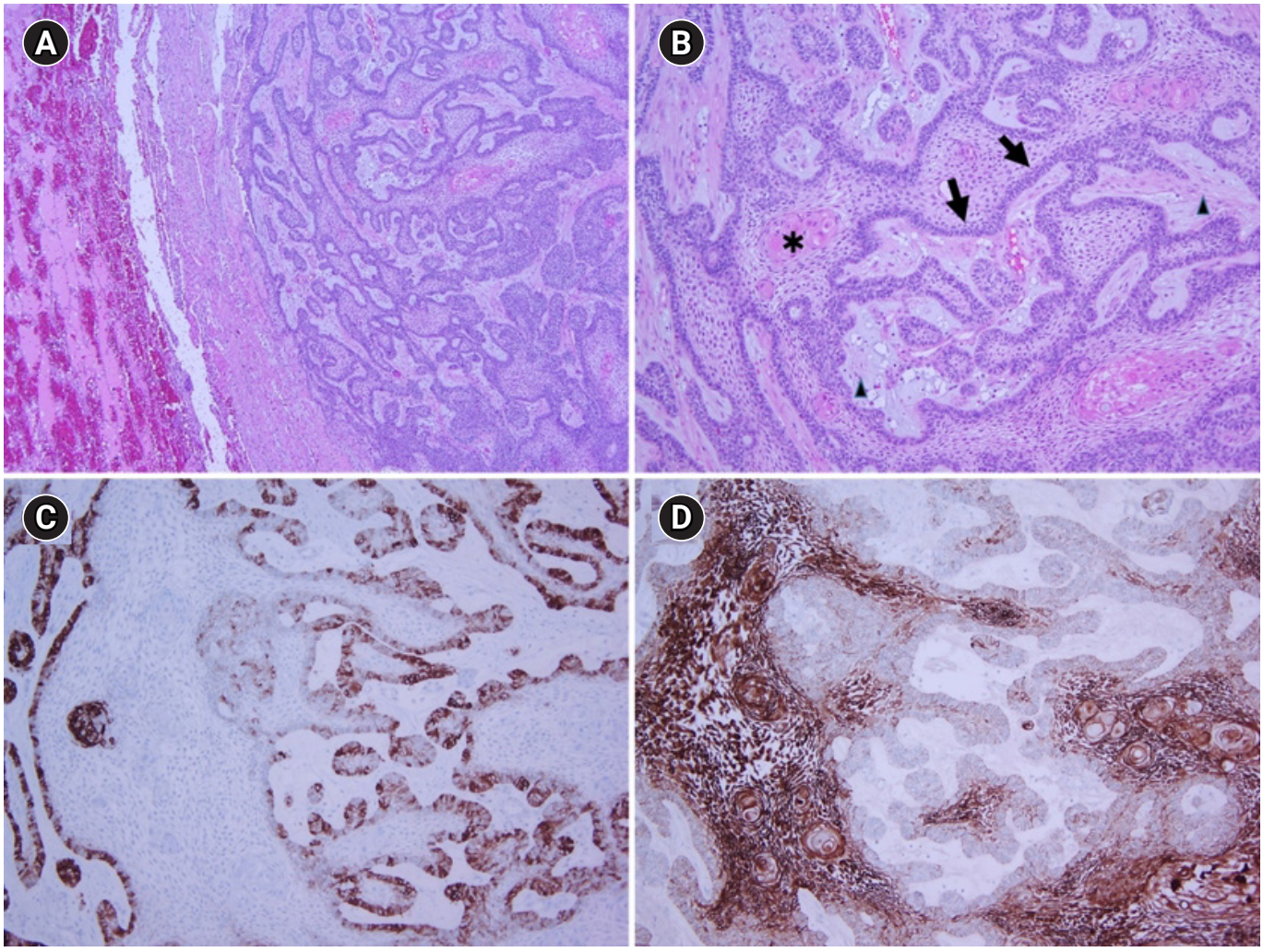

Fig. 3. Pathological findings of the lung biopsy specimen. (A) The pulmonary metastatic lesion is more cellular, with a clear margin between the lesion and the surrounding lung tissue (H&E, ×40). (B) Anastomosing epithelial strands show peripheral palisading (arrows) with more loosely arranged angular cells (arrowheads) and variable keratinization (asterisk) (H&E, ×100). (C) CD56 is preferentially expressed in peripheral cells, and (D) CK19 is expressed in all cells (immunohistochemical stain, ×100).

the first reported case of accelerated postoperative growth in metastatic ameloblastoma. There is a risk of accelerated growth of metastatic disease and increased formation of new metastatic foci in the perioperative period. However, the underlying mechanism remains unclear. There are a few hypotheses, such as surgery inducing the upregulation of adhesion molecules in target organs and recruiting immune cells capable of entrapping tumor cells [10]. Furthermore, surgery can induce changes in the target tissue and in the cancer cells to enhance migration and invasion to the target site [10]. Surgical trauma can induce local and systemic inflammatory responses, which can contribute to the accelerated growth of residual and metastatic disease [11].

Treatment of choice for primary ameloblastoma is surgical resection, while treatment for metastatic ameloblastoma remains controversial [3,12]. The evidence for treatment decisions has mostly been derived from case reports due to the disease rarity [1]. Surgical resection is the treatment for pulmonary metastasis. Chemotherapy or radiotherapy have been indicated for non-resectable lesions, but the effects are not well-studied [9,12]. Since the effects of chemotherapy are unpredictable, no single-agent or combination regimen has been recommended [1]. Radiotherapy is only for palliative care [4]. Amzerin et al. [5] reviewed the responses

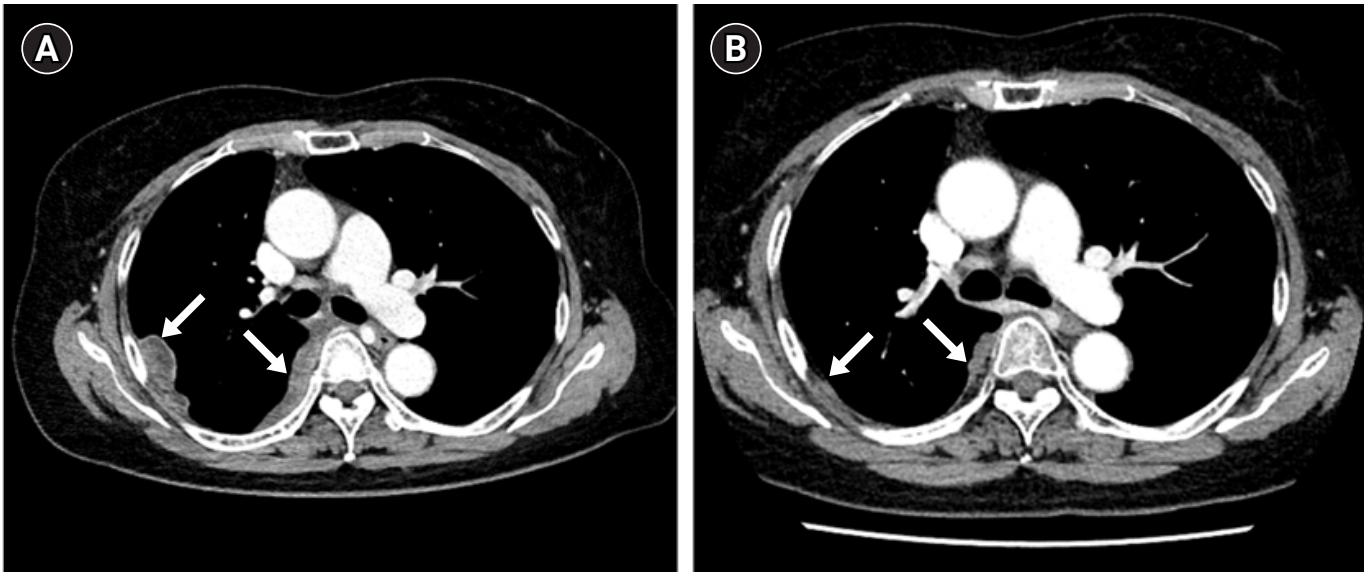

Fig. 4. (A) Chest computed tomography performed in July 2020. Newly developed multifocal enhancing pleural and fissural nodules in the right hemithorax (arrows). (B) Follow-up chest computed tomography performed after chemotherapy, showing markedly decreased size of the previously noted pleural and fissural nodules in the right hemithorax (arrows).

of chemotherapy in less than 50 cases. They recommended platinum-based regimens as first-line treatment. Therefore, we treated our patient with carboplatin and paclitaxel. She achieved partial response after six cycles. Fortunately, metastatic ameloblastoma of the lung and pleura was reduced by over 90%.

Metastatic ameloblastoma is typically asymptomatic and discovered incidentally, as in this case. The highlight of our case was the postoperative accelerated tumor growth of metastatic ameloblastoma after metastasectomy. When surgical resection was impossible, we treated with well-tolerated carboplatin and paclitaxel. In the absence of established treatment for metastatic ameloblastoma, chemotherapy using carboplatin and paclitaxel can be considered.

Article information

Conflicts of interest

No potential conflict of interest relevant to this article was reported.

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Author contributions

Conceptualization: JWJ. Data curation: JUP, JWJ. Formal analysis: JUP, JWJ. Funding acquisition: JWJ. Methodology: JUP. Project administration: JWJ. Visualization: JUP. Writing - original draft: JUP. Writing - review & editing: JUP, JWJ. Approval of final manuscript: all authors.

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Case report

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Emergency rescue stent grafting for a ruptured degenerated graft that bypassed coarctation of the aorta: a case report

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A 33-year-old man who had undergone bypass from the distal aortic arch to the proximal descending thoracic aorta with a Dacron graft to treat coarctation of the aorta 20 years prior presented to the emergency department with massive hemoptysis. He was diagnosed with a graft-to-bronchial fistula. After thoracic endovascular aneurysm repair through the coarctation, aortic replacement was performed 3 weeks later. Therefore, emergency-rescue thoracic endovascular aneurysm repair can be considered for preventing aortic rupture.

Keywords: Case reports; Coarctation of aorta; Dacron; Endovascular procedures; Ruptured aortic aneurysm

Introduction

In the literature, most late complications of Dacron grafts are anastomotic pseudoaneurysm, fistula, graft thrombosis, graft infection, or non-anastomotic aneurysmal change or rupture. Some reports on endovascular repair of Dacron graft dilatation with a subsequent anastomotic false aneurysm after post-coarctation aortic repair demonstrated the feasibility of endovascular treatment in selected candidates because it is not appropriate for all graft failure cases or for cases that require long-term follow-up for graft stability [1]. In this case, first of all we performed thoracic endovascular aneurysm repair (TEVAR) successfully to stabilize the patient's vital signs due to the risks associated with emergent aortic replacement.

Case

Ethical statements: This study was approved by the Institutional Ethics Committee of Daejeon Eulji University Hospital (EMC 2021-08-004). Informed consent was obtained from the patient.

A 33-year-old male patient was admitted with hemoptysis that measured about one liter. A bypass from the distal arch to the proximal descending thoracic aorta with a Dacron graft was achieved to treat coarctation of the aorta that had occurred 20 years prior. Additional bypass grafting from the right axillary to the femoral artery was performed 5 years

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after the initial procedure because of deterioration of coarctation. Hemoptysis originated from a graft-to-bronchial fistula, identified at a degenerative aneurysm of the Dacron graft. The diameter of the severely twisted graft on computed tomography (CT) scans was 20 mm (Fig. 1A). Although no interval change had been detected on scans obtained at 8 and 12 years, a CT scan in the emergency department revealed a diameter increase to 38 mm (Fig. 1B). TEVAR through coarctation and simultaneous extra-anatomic bypass grafting from the ascending to descending thoracic aorta were planned.

However, he presented at our hospital one day prior to admission for TEVAR due to massive hemoptysis. His medical condition was unstable. A follow-up CT scan showed increased graft size to 41 mm. We decided to perform an emergent TEVAR for coarctation of the aorta and aortic replacement when the patient's vital signs stabilized.

A 10-mm balloon angioplasty prior to TEVAR allowed the TEVAR catheter to pass the coarctation. After TEVAR using a Medtronic Valiant thoracic stent graft (22 mm in diameter, 150 mm in length; Medtronic Vascular, Santa Rosa, CA, USA) (Fig. 2A), hemoptysis was resolved. The coarctation, however, was not sufficiently relieved, and the degree of dependence on the axillofemoral bypass graft increased. After 3 weeks of recovery from pneumonia and TEVAR, the final aortic replacement was implemented after the ruptured spot on the crisp graft was located (Fig. 1C, arrow). The aorta was replaced with a 22 mm Hemashield platinum woven double velour straight graft (Maquet Getinge Group, Rastatt, Germany) (Fig. 2B). Preoperative blood pressure was 131/63 mmHg in the right radial artery and 86/52 mmHg in the descending thoracic aorta, and the postoperative values were 122/70 mmHg and 110/69 mmHg, respectively.

The patient was discharged on postoperative day 10 without complications. Postoperative, 6-month follow-up CT scan showed no abnormality (Fig. 2C).

Discussion

This case report provides two significant insights: degenerative rupture of a Dacron graft and TEVAR as a therapeutic option for aortic rupture.

Previous reports indicated that most late complications

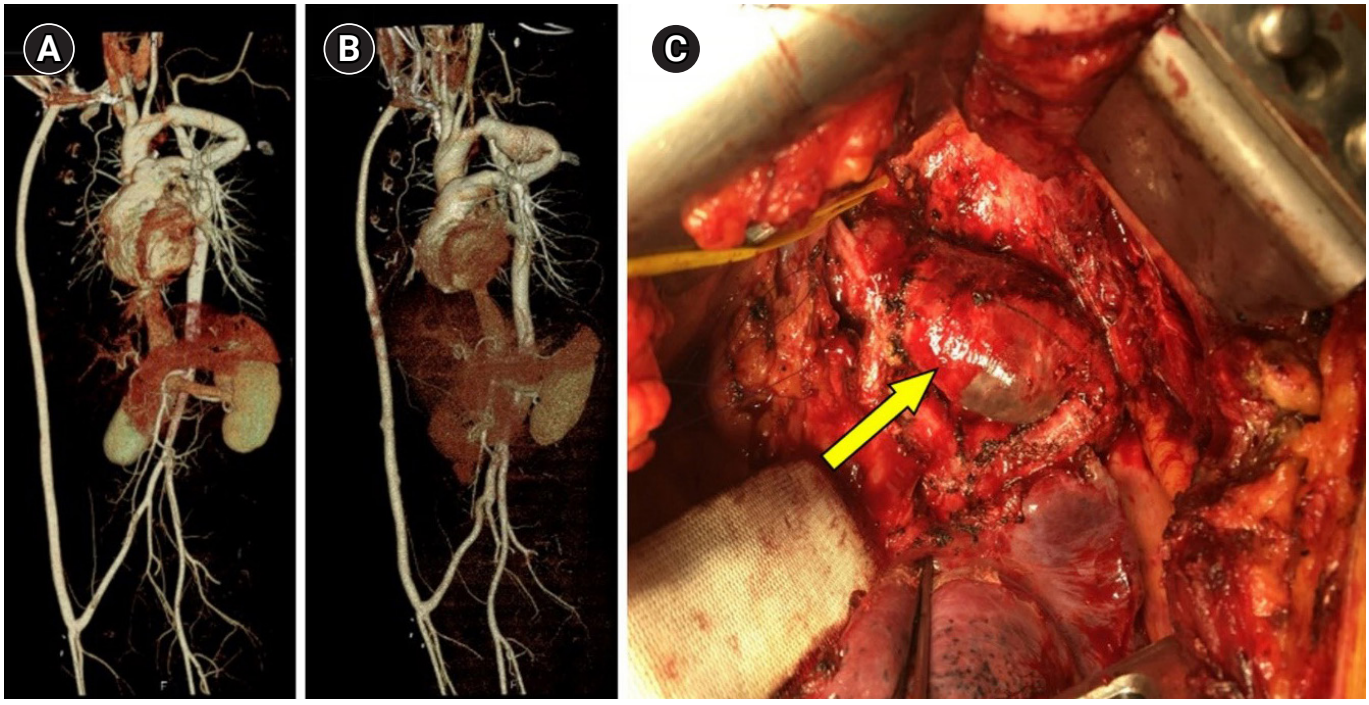

Fig. 1. Computed tomography scans from 8 years ago (A) and at presentation (B). Twisted graft with aneurysmal change at the rupture spot (arrow in C).

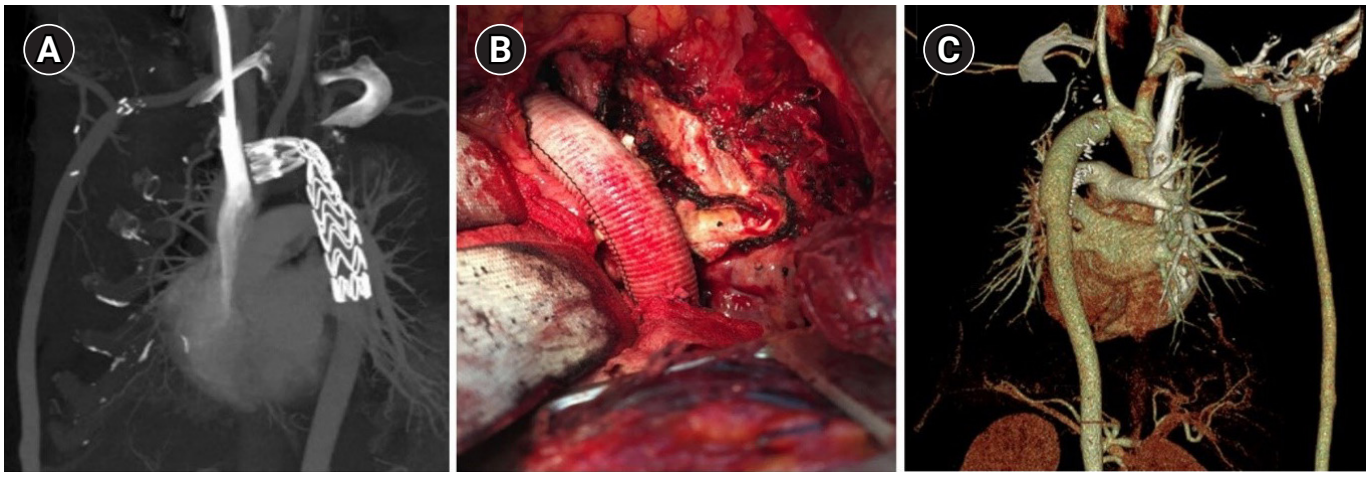

Fig. 2. Computed tomography (CT) scan after thoracic endovascular aneurysm repair, without any definite endoleak (A). The new Dacron graft in the operative findings (B) and a postoperative CT scan (C).

of Dacron grafts are anastomotic pseudoaneurysm, fistula, graft thrombosis, graft infection, or non-anastomotic aneurysmal change or rupture [1]. Complications associated with woven Dacron have been reported [2]. For this patient, the graft was in a twisted position for a long time, and the increase of afterload in the graft proximal to twisting resulted in gradual aneurysmal changes and, finally, graft rupture.

Previous reports on endovascular repair of Dacron graft dilatation with a subsequent anastomotic false aneurysm after post-coarctation aortic repair demonstrated the feasibility of endovascular treatment in select candidates; however, this approach is not appropriate for all graft failure cases or for cases that require long-term follow-up for graft stability [2].

In the present case, TEVAR was implemented to stabilize the patient's vital signs [3] due to the risks associated with emergent aortic replacement [4].

Recent reports have indicated that systolic pressure gradients were reduced significantly to 4±6 mmHg after TEVAR [5]. In the present case, however, the systolic pressure gradient was 45 mmHg (131 mmHg vs. 86 mmHg), and the internal diameter was less than 10 mm. Therefore, the next aortic replacement was planned based on patient age, risk of progressive cardiac afterload or cerebral hypertension, and delayed complications of TEVAR.

Article information

Conflicts of interest
No potential conflict of interest relevant to this article was reported.

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Author contributions
Conceptualization: JHC, MSC, HL. Data curation: JHC, MSC, HL. Formal analysis: JHC, MSC, HL. Methodology: JHC, HL. Project administration: JHC, MSC, HL. Visualization: MSC. Writing-original draft: JHC, MSC, HL. Writing-review & editing: JHC, HL. Approval of final manuscript: JHC, MSC, HL.

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- The title of the secondary publication should indicate that it is a secondary publication (complete or abridged republication or translation) of a primary publication. Of note, the US National Library of Medicine (NLM) does not consider translations to be "republications" and does not cite or index them when the original article was published in a journal indexed in MEDLINE.

Conflict of interest statement

The corresponding author must inform the editor of any potential conflicts of interest that could influence the authors' interpretation of the data. Examples of potential conflicts of interest are financial support from or connections to pharmaceutical companies, political pressure from interest groups, and academically related issues. In particular, all sources of funding applicable to the study should be explicitly stated.

Commercial sponsorships or funding

The corresponding author must inform the editor of any commercial sponsorships or fundings.

Registration of the clinical trial research

A clinical trial defined as “any research project that prospectively assigns human subjects to intervention and comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome” should be registered with the primary registry prior to publication. The KMJ accepts registration in any of the primary registries that participate in the WHO International Clinical Trials Portal (https://www.who.int/clinical-trials-registry-platform), NIH ClinicalTrials.gov (http://www.clinicaltrials.gov), ISRCTN Resister (www.ISRCTN.org), or the Clinical Research Information Service (CRIS), Korea CDC (https://cris.nih.go.kr/cris/index.jsp). The clinical trial registration number shall appear at the end of the abstract.

Data-sharing statement

The KMJ accepts the “ICMJE Recommendations for Data Sharing Statement Policy.” All manuscripts reporting clinical trial results from July 1, 2018 should submit a data-sharing statement following the ICMJE guidelines. Authors can refer to the editorial, “Data Sharing Statements for Clinical Trials: A Requirement of the International Committee of Medical Journal Editors,” in JKMS Vol. 32, No. 7:1051-1053 (http://crossmark.crossref.org/dialog/?doi=10.3346/jkms.2017.32.7.1051&domain=pdf&date_

stamp=2017-06-05).

Statement of informed consent and IRB approval

Copies of written informed consent and institutional review board (IRB) approval for clinical research (original article and case report) should be kept. Authors should include the IRB approval number in the manuscript. If necessary, the editor or reviewers may request copies of these documents to resolve questions about IRB approval and study conduct. For studies involving human subjects, the method through which informed consent was obtained from the participants must be stated in the Methods section.

Statement on human and animal rights

Clinical research should be done in accordance with the Ethical Principles for Medical Research Involving Human Subjects, as outlined in the Helsinki Declaration (https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/). Clinical studies that do not meet the Helsinki Declaration will not be considered for publication. For publication, the human subjects' identifiable information, such as the patients' names, initials, hospital numbers, dates of birth, or other protected healthcare information should not be disclosed. For animal subjects, the research should be performed based on the National or Institutional Guide for the Care and Use of Laboratory Animals, and the ethical treatment of all experimental animals should be maintained.

Process to manage the research and publication misconduct

When the Journal faces suspected cases of research and publication misconduct, such as a redundant (duplicate) publication, plagiarism, fabricated data, changes in authorship, undisclosed conflicts of interest, an ethical problem with a submitted manuscript, a reviewer who has appropriated an author's idea or data, complaints against editors, and other issues, the resolution process will follow the flowchart provided by the Committee on Publication Ethics (http://publicationethics.org/resources/flowcharts). The Editorial Board of the KMJ will discuss the suspected cases and reach a decision. The KMJ will not hesitate to publish errata, corrigenda, clarifications, retractions, and apologies when needed.

Errata

Errata provide a means of correcting errors that occur during the writing, typing, editing, or publication (e.g., a misspelling, dropped word or line, or mislabeling of a figure) of a published article. Errata can be submitted through the KMJ online manuscript management portal as a Microsoft Word file. In the Abstract section of the submission form (a required field), enter "Not Applicable" and upload the text of the proposed erratum. Please see a recent issue for correct formatting.

Author corrections

If the addition or deletion of authors or a change to the order of authorship is required, the corresponding author must complete the authorship change form and submit it through the KMJ online manuscript management portal with the signature of all existing authors and new authors. In the Abstract section of the submission form (a required field), enter "Not Applicable." The text of the proposed erratum should be uploaded as a Microsoft Word file. When there is a request for a change by the author, the editorial committee assembles an ethics committee to judge whether the change is appropriate. If a new author should be added or an author should be deleted after submission, it is the responsibility of the corresponding author to ensure that all authors are aware of and agree to the change in authorship. The KMJ assumes no responsibility for such changes. Please see a recent issue for correct formatting.

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Retractions should be submitted through the KMJ online manuscript management portal as a Microsoft Word file. In the Abstract section of the submission form (a required field), put "Not Applicable." Letters of agreement signed by all of the authors must be supplied as Miscellaneous Files Not for Publication (scanned PDF files). The retraction will be assigned to the journal's editor-in-chief, and the editor who handled the paper and the chair of the KMJ editorial board will be consulted. If all parties agree to the publication and content of the retraction, it will be sent to the journals department for publication.

For the policies on research and publication ethics not stated in the Instructions, Guidelines on Good Publication

(http://publicationethics.org) or Good Publication Practice Guidelines for Medical Journals (http://kamje.or.kr) can be applied.

V. MANUSCRIPT PREPARATION AND FORMAT

1. General requirements

- Every manuscript should be written in English.
- The main document, including manuscript text and tables, should be prepared in MS Word.
- The manuscript should be double-spaced on 21.6 × 27.9 cm (US letter) or 21.0 × 29.7 cm (A4) paper with top, bottom, right, and left margins of 3.0 cm.
- All manuscript pages are to be numbered consecutively, beginning with the abstract as page 1. Neither the authors' names nor their affiliations should appear on the manuscript pages.
- Use only standard abbreviations; use of nonstandard abbreviations can be confusing to readers. Avoid abbreviations in the title of the manuscript. A spelled-out term followed by the abbreviation in parentheses should be used on first mention unless the abbreviation is widely recognized (e.g., UN for United Nations).
- The names and locations (city, state, and country) of manufacturers of equipment and non-generic drugs should be given.
- When quoting from other sources, supply a reference number after the author's name or at the end of the quotation (see References section).
- Authors should express all measurements in conventional units, using the International System (SI) of units.
- To make papers more readable and informative, mark the followings in italics.
  - Biological names of organisms: *Saccharomyces cerevisiae*, *E. coli*
  - Restriction enzymes and some enzymes: *EcoRI, Taq polymerase*
  - Names of genes: *src, c-H-ras, myc*
  - Latin words: *in vivo, in vitro, in situ*
  - Centrifugal acceleration: *g* (e.g., 100,000 *g*)

2. Reporting guidelines for specific study designs

Research reports frequently omit important information. As such, reporting guidelines have been developed for a number of study designs, and some journals may ask authors to follow them. Authors are also encouraged to consult the reporting guidelines relevant to their specific research design. A good source for reporting guidelines is the EQUATOR Network (http://www.equator-network.org/home) and the United States National Library of Medicine (http://www.nlm.nih.gov/services/research_report_guide.html).

3. Original articles

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The manuscript should be prepared according to the "ICMJE Recommendations for the Conducts, Reporting, and Publication of Scholarly Work in Medical Journals" (2021) (http://www.icmje.org). In addition to the ICMJE recommendation, a number of reporting guidelines have been developed by experts to facilitate reporting of research studies or clinical trials (http://www.equator-network.org/library). For reporting of randomized controlled trials, the KMJ requires compliance with the CONSORT statement (http://www.consort-statement.org) and the ICMJE Statement on Data Sharing (http://www.icmje.org/recommendations/browse/publishing-and-editorial-issues/clinical-trial-registration.html).

Manuscript components

The manuscript, including tables and their footnotes and figure legends, should use the 12-point Times New Roman font and line spacing should be set to "double." The manuscript should be in the following sequence: title page, Conflicts of interest, Sources of funding, Author contributions, ORCID iDs, Abstract and Keywords, Introduction, Methods, Results, Discussion, Acknowledgments, References, Tables (each table complete with a title and footnotes), and Figure Legends (figures should be uploaded separately, not embedded in text). All pages should be numbered consecutively starting from the title page. All numbers should be written in Arabic numerals throughout the manuscript except for the first word of a sentence. Tables and figures should not be inserted in the main text. A combined table file can be uploaded separately from the main manuscript file. A combined figure file or individual figure files can be uploaded separately from the main manuscript.

File formats

Drawings, photographs, graphs, or combined figures can be

submitted as PDF files. We accept graphic files in the PDF, JPEG, or TIFF formats only. It is permissible to send low-resolution images for peer review, but we will ask for high-resolution files before publication. Acceptable file formats for supplemental video files are mov, wmv, mpg, mpeg, and mp4. The file size and running time of each video should be no more than 25 MB and 5 minutes, respectively.

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Include the following items on the title page: (1) the title of the manuscript (the title should be short, informative, contain the major keywords, and be no more than 200 characters in length, including spaces between words; acronyms and abbreviations should be avoided, and the species of any experimental animal must be indicated in the title; (2) an author list (include ORCID*); (3) the names of each author's institutional affiliation; (4) the name, address, telephone number, and email address of the corresponding author; (5) the source of any research funding, if any, and a list of where and when the study has been presented in part elsewhere; and (6) a running title of no more than 50 characters.

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Author contributions

What authors have contributed to the study should be described in this section. To qualify for authorship, all contributors must meet at least one of the 12 core contributions defined by CRediT (conceptualization, methodology, software, validation, formal analysis, investigation, data curation, funding acquisition, project administration, resources, supervision, and visualization), as well as at least one writing contribution (original draft preparation, review, and editing). Authors may also satisfy the other remaining contributions; however, these alone will not qualify them for authorship.

Contributions will be published with the article, and they should accurately reflect contributions to the work. The submitting author is responsible for completing this information at submission, and it is expected that all authors will have reviewed, discussed, and agreed to their individual contributions ahead of this time.

An example:

Conceptualization: GDH. Data curation: YSJ, YC. Formal analysis: CSK. Methodology: YK, GDK.; Software: YK, YSJ. Validation:..... Investigation:..... Funding acquisition:..... Project administration:..... Resources:..... Supervision:..... Visualization:..... Writing - original draft: GDH, YSJ. Writing - review and editing: YC, YK, CSK.

Abstract and keywords

The abstract should briefly describe the content of the manuscript in a structured format within 250 words. The abstract should be structured as follows; Background, Methods, Results, and Conclusion. Initialisms, acronyms and informal abbreviations should be avoided where possible; if needed, they should be kept to an absolute minimum and accompanied by proper definitions. Three to five keywords should be listed below the Abstract on the Abstract page. For the selection of keywords, refer to Medical Subject Headings (MeSH) in PubMed, or http://www.nlm.nih.gov/mesh/MBrowser.html.

Main text

- Introduction

A brief background, references to the most pertinent papers general enough to inform readers, and the relevant findings of others should be included. It is recommended that the introduction include a "General and specific background," "Debating issue," and "Specific purpose of this study."

- Methods

The explanation of the experimental methods used should be concise and sufficient for repetition by other qualified investigators. Procedures that have been published previously should not be described in detail. However, new or significant modifications of previously published procedures require full descriptions. Clinical studies or experiments using laboratory animals or pathogens should mention approval of the studies by relevant committees in

this section. The sources of special chemicals or preparations should be supplied, along with their location (name of company, city and state, and country). The method of statistical analyses and the criteria for determining significance levels should be described. An ethics statement should be placed in this section when the studies are performed using clinical samples or data, and animals. Examples are provided below.

(For clinical study)

The present study protocol was reviewed and approved by the Institutional Review Board of xxx XXXXXX of the University College of Medicine (approval No. 2018001). Informed consent was submitted by all subjects when they were enrolled.

(For animal study)

The procedures used and the care of animals were approved by the Institutional Animal Care and Use Committee at xxxXXXXX University (approval No. 2018002).

(For clinical trials)

This was a randomized clinical trial at the second phase, registered at the Clinical Research Information Service (CRIS, http://cris.nih.go.kr), number KCT0002018.

Other international registration is acceptable. Manuscripts reporting interventional clinical trial should include a data-sharing plan following the ICMJE statement by referring to the ICMJE Statement on Data Sharing.

When describing participants, ensure correct use of the terms sex (for biological factors) and gender (identity, psychosocial, or cultural factors), and, unless inappropriate, report the sex or gender of study participants, the sex of animals or cells, and describe the methods used to determine sex or gender. If the study involved an exclusive population, (e.g., only one sex), authors should justify why, except in obvious cases (e.g., prostate cancer). Authors should describe how they determined race or ethnicity and justify their relevance.

• Results

This section should be presented logically and in a manner consistent with the Methods section. Tables and figures are recommended when they can present data more succinctly and clearly. Do not duplicate the content of tables or figures in the Results section. Briefly describe the core results related to the conclusion in the text when data are provided in tables or in figures. In the Results section, audio or video files are also welcomed. Supplementary results can be placed in an Appendix.

• Discussion

The data should be interpreted concisely without repeating materials already presented in the Results section. A summary or conclusion should be included at the end of this section. We recommend authors describe the clinical or biomedical significance of the study. Speculation is permitted but must be clearly supported by the results or literature published.

Acknowledgments

The authors can list the names of persons who helped plan or conduct the study but are not eligible authors in this section. Funding sources, which are supplied on the title page, should not be duplicated in this section.

References

References should be numbered in the order they appear in the text and should not exceed 40 in number. Citation of references in the text should be identified with Arabic numerals in square brackets (for example, ... the leading cause of death in Korea [1,2] and preceding any punctuation). References should be listed in the order they are cited in the text, with consecutive numbers in this section. The style for citing papers in periodicals is: last name and initials of all authors, full title of article, journal name abbreviated in accordance with the PubMed style, year, volume, issue number and first and last page numbers. The style for a chapter of a book is: author and title of the chapter, editor of the book, title of the book, edition, volume, place (city), publisher, year, and first and last page numbers. Up to six authors can be listed in a reference; the names of all authors after the first 6 should be abbreviated to "et al." The use of software to manage references (e.g., EndNote) is recommended.

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   Verbalis JG. Renal physiology of nocturia. Neurourol Urodyn 2014;33(Suppl 1):S6-9.
2. Book
   Hong GD, Kim C, Park J. KMJ reference style: a guide for authors. 5th ed. Seoul, Korea: Daehakro Press; 2017.
3. Chapter in a book
   Floch MH. Probiotics, probiotics and dietary fiber. In: Buchman A, editor. Clinical nutrition: a guide for gastroenterologists. Thorofare, NJ: SLAK Incorporated; 2005. p. 18-24.
4. Conference/meeting presentation
   Morrow GR, Ryan JL, Kohli S. Modafinil for persistent post-treatment fatigue: an open label study of 82 women with breast cancer. MASCC international symposia 2006, Abstract 11-070.
5. Internet or electronic resource
   Testa J. The Thomson Reuters journal selection process [Internet]. Philadelphia: Thomson Reuters; c2012 [cited 2013 Sep 30]. Available from: http://wokinfo.com/essays/journal-selection-process

Tables and figures

Tables: Tables are to be numbered in the order in which they are cited in the text. A table title should concisely describe the content of the table so that a reader can understand the table without referring to the text. Each table must be simple and provided on a separate page with its title. Explanatory matter is placed in the footnotes below the table and not in the title. All non-standard abbreviations should be explained in the footnotes. The symbols a), b), c), d), should be used (in that order) for footnotes. Statistical measures such as standard deviation (SD) or standard error (SE) should be identified. No vertical or horizontal rules should appear between table cells.

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Figures should be numbered, using Arabic numerals, in the order in which they are cited. In the case of multiple prints bearing the same number, use English letters immediately after the numerals to indicate the correct order. (e.g., Fig. 1A, Fig. 1B and C; Fig. 2A–D).

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Description of *p* value: p is always italicized and in lower case. When p values are statistically significant, the corresponding data should be marked with superscripted asterisks ($p<0.05$, $p<0.01$, $p<0.001$).

Abbreviations: Except for units of measurement, abbreviations are strongly discouraged. Do not use abbreviations in the title or abstract and limit their use in the text. Define all abbreviations at first mention.

Units of measurement: Laboratory values are expressed using conventional units of measure, with relevant System International (SI) conversion factors, if necessary expressed secondarily (in parentheses) only at first mention. Figures and tables should use conventional units, with conversion factors given in legends or footnotes. The metric system is preferred for the expression of length, area, mass, and volume.

Names of drugs, devices, and other products: Generic names of pharmaceuticals should be used. When proprietary brands are used, include the brand name and the name of the manufacturer in parentheses after the first mention of the generic name in the Methods section.

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Supplementary materials

Authors can submit supplementary materials for online-only publication when there is insufficient space to include the materials in the main article. Supplementary materials should be original and important to the understanding and interpretation of the report. As supplementary materials will not be edited or formatted after publication, authors are responsible for the accuracy and presentation of this material.

Supplementary materials should be submitted in a single MS Word document or a single PDF file, which should include all materials (information, tables, figures, and references). Each element included in supplementary material should be cited in the text of the main manuscript (e.g., Supplementary Table 1, Supplementary Fig. 1, Supplementary Methods). The first page of the online-only document should list the number and title of each element included in the document.

4. Review articles

Reviews are comprehensive analyses of specific topics. They are typically solicited by the Editor, but we also consider unsolicited material. Please send us a Presubmission Inquiry before writing a review article. All review articles undergo the same review process as other types of articles prior to acceptance. Reviews are organized as follows: title page, abstract (a single unstructured paragraph of no more than 250 words) and three to five keywords (as in MeSH), introduction, main text, conclusion, acknowledgments, references, tables, and figure legends. Reviews have no restrictions on word count or the number of figures and tables. However, authors should eliminate redundancies, emphasize the central message, and provide only the data necessary to convey that message. A review article is limited to less than 6,000 words, with a maximum of 100 references, excluding tables or figure legends.

5. Case reports

Case reports will be published only in exceptional circumstances, if they illustrate a rare occurrence of clinical importance. The manuscript should be in the following sequence: title page, abstract (no more than 250 words) and three to five keywords (as in MeSH), introduction, case report(s), discussion, acknowledgments, references (no more than 20), tables, and figure legends. The manuscript should have no more than six tables or figures in total. For case reports involving humans, whether informed consent for publication of clinical data was obtained from the study participants must be stated in the case description section.

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Editorials are invited by the Editor and should provide commentary on articles in the current issue. Editorial topics could include active areas of research, fresh insights, and debates in all fields of interest to KMJ readers. Editorials should not exceed 1,000 words, excluding references, tables, and figure legends. No more than 20 references should be cited. The manuscript should have no more than three tables or figures. Any article longer than these limits should be discussed with the editor.

Table 1. Specifications for publication types

Type of article	Original article	Review article	Case report	Editorial
Abstract (words)	250	250	250	NR
Text (words)[a]	NL	6,000	1,500	1,000
References	40[b]	100	20	20
Tables and Figures	NL	NL	6	3

NL, not limited; NR, not required.
[a]Maximum number of words is exclusive of the abstract, references, and figure legends.
[b]Except meta-analyses or systematic reviews.

VI. INSTRUCTIONS FOR SUBMISSION OF REVISED MANUSCRIPTS

To prepare a revised version of your manuscript, authors should carefully follow the instructions given in the Editor's letter. Failure to do so will delay a decision on a revision. If references, tables, or figures are moved, added, or deleted

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Changes referring to an Editor or reviewer query regarding moving or adding references, tables, or figures should be highlighted. Deletions should be marked with strikethrough (a line drawn through the selected text, using the ab button) and highlighted. Authors should submit a highlighted clean version without using the track changes function in MS Word.

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☐ **Title page:** (1) The title of the manuscript; (2) author list (include ORCID); (3) names of each author's institutions and an indication of each author's affiliation; (4) the name, address, telephone numbers, and e-mail address of the corresponding author; (5) if necessary, the source of any research funding; and (6) a running title of fewer than 50 characters.

☐ **Abstract and Keywords**

- Original article (structured—background, methods, results, and conclusion—within 250 words)
- Review article (unstructured, ≤250 words)
- Case report (unstructured, ≤250 words)

Three to five keywords as in MeSH

☐ **Main text**

- Original article (introduction, methods, results, discussion)
- Review article (≤6,000 words), Case report (≤1,500 words), Editorial (≤1,000 words)

Turn on serial line numbering from the beginning of the main text.

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Check that all references in the References section are cited in the text and vice versa.

- Original article (≤40 references)
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All table and figure numbers found in the text. No incorrectly numbered sections and mislabeled tables or figures must be found.

Each figure as separate files, in JPEG, TIFF, PDF format, minimum 300 dpi.

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☐ **Title page:** (1) The title of the manuscript; (2) author list (include ORCID); (3) names of each author's institutions and an indication of each author's affiliation; (4) the name, address, telephone numbers, and email address of the corresponding author; (5) if necessary, the source of any research funding; and (6) a running title of fewer than 50 characters.

☐ **Abstract and Keywords**

· Original article (structured—background, methods, results, and conclusion—within 250 words)
· Review article (unstructured, ≤250 words)
· Case report (unstructured, ≤250 words)
Three to five keywords as in MeSH

☐ **Main text**

· Original article (introduction, methods, results, discussion)
· Review article (≤6,000 words), Case report (≤1,500 words), Editorial (≤1,000 words)
Turn on serial line numbering from the beginning of the main text.

☐ **References**

Check that all references in the References section are cited in the text and vice versa.
· Original article (≤40 references)
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All table and figure numbers found in the text. No incorrectly numbered sections and mislabeled tables or figures must be found.
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- See the Authorship Policy in Instructions for Authors.

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- Methodology: _____
- Software: _____
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