Aims and scope

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We spend 25 to 30 years sleeping in our lifetime. This time-consuming activity plays an important role in the quality of life. Chronic sleep disturbance is a pandemic worldwide, with two-thirds of individuals unable to obtain the recommended 7 to 9 hours of sleep each night. In addition, up to 10% of people meet the criteria for clinical insomnia [1]. The cost of insomnia is estimated to reach $100 billion annually in the United States alone. Therefore, in this special issue, we attempt to understand the importance of sleep from several aspects: (1) understanding sleep, which affects not only mental health but also overall physical health [2]; (2) understanding the characteristics of sleep disturbances that occur in autism spectrum disorder (ASD) mainly in children [3]; and (3) new non-pharmacological treatments for insomnia [4].

Insomnia is treated as a neuropsychiatric disorder and its influence was thought to be confined to neurocognitive and emotional problems. However, insomnia affects the entire body and causes a variety of physical illnesses. Yun and Jo [2] reviewed the aspects of insomnia as a systemic disease. Chronic sleep disturbances affect a variety of physiological systems, including the immune, endocrine, and circulatory systems. A negative impact on these systems increases the risk of various illnesses, such as diabetes, high blood pressure, cancer, and infections. Accordingly, Yun and Jo [2] argued that insomnia should be considered a systemic disease, and they described in detail the complex, dynamic, and global biological nature of the sleep process.

Sleep plays an important role in adults but is also important for child development. Sleep is a necessary physiological process for typical synaptic development and brain maturation, and children with sleep disturbances are vulnerable to emotional and cognitive developmental problems [5]. Seo [3] reviewed in detail the sleep disturbances seen in ASD among the neurodevelopmental disorders that mainly occur in children. The developmental and behavioral effects of sleep disturbances in ASD were examined in detail, and the underlying mechanisms were investigated. In addition, he looked at the therapeutic approaches to sleep disturbances in children and adolescents with ASD, including pharmacological and non-pharmacological treatments.

Nevertheless, new attempts are being made to treat sleep disorders. Sleep medications commonly used for sleep disorders have many side effects and therapeutic limitations. Kim [4] reviewed the effects of mindfulness meditation, which has recently been in the spotlight, on insomnia. Patients with chronic insomnia describe their condition as a “vicious cycle.” In other words, insomnia worsens as the effort and desire to sleep increases. These cognitive distortions and excessive sleep obsessions cannot be resolved with medication alone. The concept and therapeutic mechanism of mindfulness meditation that is helpful in patients...
with chronic insomnia will be examined in detail.

I hope these articles will help clinicians and scientists better understand sleep and insomnia. These articles are expected to be helpful for a comprehensive understanding of sleep. The systemic characteristics of sleep were examined through physiological mechanisms, and the sleep patterns of children with ASD, which have been addressed little in the past, were examined in depth. A mindfulness-based insomnia treatment, a non-pharmacological treatment that has recently been in the spotlight, was also described in detail.

Finally, I would also like to thank the Editorial Board of Yeungnam University Journal of Medicine for giving us the opportunity to conduct a multifaceted review of sleep. I appreciate the hard work of the authors of these articles.

Notes

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References

Understanding insomnia as systemic disease

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Introduction

Insomnia is a disease in which there is a problem with the quality or quantity of sleep. According to Diagnostic and Statistical Manual of Mental Disorders (5th edition), difficulty in initiating sleep, difficulty in maintaining sleep, and frequent awakenings or problems returning to sleep after awakenings were described as major symptoms of insomnia [1]. The prevalence of insomnia is reported to be 2.3% to 25.5% in international studies [2]. However, the 1-year incidence rate of simple insomnia symptoms is 30.7% [3]. Direct and indirect costs due to insomnia have been investigated to reach $100 billion per year in the United States alone [4]. Past history, family history, increased arousability, poor general health, and higher bodily pain are known risk factors for insomnia [3]. Insomnia is also a major psychiatric disorder, often comorbid with various psychiatric disorders, and is also a symptom of some psychiatric disorders [5]. Because of these characteristics, insomnia is treated as a neuropsychiatric disorder, and the effects of insomnia tend to be limited to the neurocognitive and affective domains. However, insomnia is known to affect physiological processes that correspond to a wide range of areas of the body and cause various physical ailments [6]. Therefore, in this review, we will examine the aspects of insomnia as a systemic disease and explain why insomnia has a wide range of effects on mental and physical health in relation to the role that sleep plays in our bodies.

Insomnia and cognitive function

The negative effects of insomnia on daytime cognitive function are well-known, and many studies have been conducted [7]. Insomnia is known to affect various cognitive domains such as memory, concentration, and attention [8].

Since sleep itself plays an important role in memory consolidation, many studies have been conducted on the effect of insomnia...
However, since other studies did not confirm the change in work-
treal Cognitive Assessment (MoCA) scores were lower in the in-
that naming, immediate memory, delayed recall, and overall Mon-
conducted with various studies recently. Guo et al. [22] confirmed
results tend to be inconsistent; therefore, a meta-analysis has been
apostate issue compared to other cognitive domains, and the re-
association between executive function and insomnia has recently become an
and shifting attention previously introduced [21]. The association
function is a set of neurocognitive skills involved in
problem-solving, including working memory, inhibitory control,
shifting/flexibility. Therefore, executive function is a cognitive
domain that includes some concepts, such as working memory
and shifting attention previously introduced [21]. The association
between executive function and insomnia has recently become an
apostate issue compared to other cognitive domains, and the re-
results tend to be inconsistent; therefore, a meta-analysis has been
conducted with various studies recently. Guo et al. [22] confirmed
that naming, immediate memory, delayed recall, and overall Mont-
treal Cognitive Assessment (MoCA) scores were lower in the in-
omnia group using the Pittsburgh Sleep Quality Index and
MoCA. They also found that an increase in the severity of insom-
nia translates to a more extensive and severe cognitive impairment.
Fortier-Brochu and Morin [23] found that there was an impair-
ment of episodic memory and attention in the insomnia group,
which was associated with sleep continuity, sleep microstructure,
and dysfunctional belief. Pulda and Schulz [24] confirmed that
there was impairment in episodic memory, problem-solving, ma-
nipulation in working memory, and retention in working memory
through a meta-analysis of 24 insomnia studies, but the degree of
impairment remained small to moderate. They, in a meta-analysis
of 18 insomnia studies, found no consistent evidence of cognitive
dysfunction associated with insomnia. They noted that the differ-
ence in sample size and situational factors may have an effect as the
sample size and situational factors produce different results in dif-
ferent studies. As such, impairment of executive function due to in-
somnia tends to have different results even in a meta-analysis;
therefore, further evaluation is necessary.

Insomnia and emotion regulation

Sleep and emotion have bidirectional interactions with each other
[25]. Therefore, chronic insomnia is known to affect emotion reg-
ulation [26]. Several studies have reported that sleep depriv-
due to insomnia affects emotional valence; hence, poor sleep quality
induces low positive emotions and high negative emotions, and this
tendency is reported to be stronger in women [27]. However,
in another study, this effect on sex was not confirmed [28]. Emo-
tional disturbances caused by sleep deprivation are known to in-
volve the amygdala and the medial prefrontal cortex [29]. It is also
known that insomnia, negative emotions, and intrusive or unwant-
ed thoughts tend to aggravate symptoms by composing a vicious
cycle with each other [30]. A study by Markarian et al. [31] found
that emotional dysregulation difficulties were associated with de-
pression, anxiety, and stress and that sleep quality plays an import-
ant role. Another study found that aggression and impulsivity are
associated with worse sleep quality and higher insomnia scores
[32]. It has been reported that emotional dysregulation due to in-
somnia is associated with changes in heart rate variability (HRV).
These results suggest that the mechanism by which insomnia caus-
es emotional dysregulation is closely related to brain-body interac-
tions known to reflect HRV [33]. Interoception is a concept that
includes nociception, thermoception, and visceral sensation and is
known to play an important role in brain-body interactions. Intero-
ception was also confirmed to have profound effects on insomnia
[34]. Heartbeat evoked potential (HEP) is a heartbeat-induced
change in the electroencephalogram (EEG) signal that is known to
directly reflect interoceptive processing. Changes in HEP have
been reported in insomnia patients, suggesting that insomnia may
be closely related to changes in interoception. [35]. In addition, in
recent studies, interoception has been shown to play an important
role not only in emotion regulation but also in anxiety and depres-
sion induced by sleep deprivation [36]. Recent discoveries on in-
teroception have given strength to the hypothesis that changes in
interoception due to insomnia are a key mechanism in the develop-
ment of symptoms such as emotion regulation disturbance, anx-
xiety, and depression [37].
Insomnia and neuropsychiatric disorders

Insomnia not only affects cognitive function and emotion regulation but is also strongly associated with several related neuropsychiatric disorders [38]. Depression and insomnia have a bidirectional relationship [39]. In a 40-year longitudinal follow-up study of medical students, it was found that individuals with baseline insomnia were twice as likely to develop the major depressive disorder than those who did not [40]. In addition, the odds ratio that an insomnia patient without depression would later develop depression was 6.2, and the odds ratio that a depressed patient without insomnia would later develop insomnia was 6.7 [41]. Insomnia is also known to increase the risk of suicidal ideation in subjects without mental disorders [42,43].

Insomnia is known to increase the risk of various anxiety disorders and depression. In a population-based study of 2,393 random individuals, symptoms of insomnia related to anxiety disorders were identified as short sleep duration, daytime sleepiness, and sleep disturbance [41]. Among anxiety disorders, generalized anxiety disorder is a disease closely related to insomnia as there are sleep disturbance symptoms in the main symptoms [1]. In individuals reporting symptoms of insomnia in a French population study, generalized anxiety disorder was the most prevalent psychiatric disorder [44]. Various anxiety disorders, such as panic disorder, specific phobia, and social anxiety disorder, have been reported to be comorbid with insomnia [41].

The cross-sectional relationship between dementia and insomnia has been well-known for a long time, but recently, various cohort studies and meta-analysis studies have reported that insomnia increases the risk of all types of dementia [45]. In a nationwide population-based study in Taiwan, insomnia was found to increase the risk of Alzheimer dementia, especially if insomnia started before the age of 40 years [46]. There are several possible mechanisms by which chronic insomnia increases the risk of dementia. The hyperarousal state caused by insomnia induces overactivation of the hypothalamic-pituitary-adrenal cascade, leading to neurodegeneration [47]. In addition, aggregation of β-amyloid in the brain is considered the main cause of Alzheimer dementia. β-amyloid is actively excreted during sleep, and a decrease in β-amyloid clearance due to insomnia is also thought to increase the risk of dementia [47]. However, there is controversy regarding the amyloid hypothesis as a pathophysiology of dementia. Therefore, additional research is needed to elucidate the exact reason why insomnia increases the risk of dementia.

Insomnia and physiological process

Insomnia is also known to cause various physiological changes during the day and night. Monroe [48] found elevated rectal temperature, heart rate, basal skin resistance, and phasic vasoconstriction in poor sleepers. It is known that these changes in the autonomic nervous system are caused by an imbalance between the sympathetic and parasympathetic nervous systems [49]. Imbalance of the autonomic nervous system, which is the main pathogenesis of insomnia, is also a key mechanism in the hyperarousal model, the main pathogenesis of insomnia [50].

Since various hormones, such as growth hormone and melatonin, are involved in sleep regulation, sleep and hormones have a reciprocal relationship [51]. Therefore, insomnia causes dysregulation of various endocrine systems [52]. An increase in cortisol hormone and a change in the 24-hour metabolic rate have been observed in patients with chronic insomnia [13,53]. In addition, it was confirmed that sleep deprivation decreased insulin sensitivity, while insulin resistance and secretion increased, especially in elderly patients with insomnia [54,55]. Insomnia is also known to induce changes in the endocrine system that controls appetite. Limited total sleep time was associated with higher ghrelin and reduced leptin levels, according to the Wisconsin Sleep Cohort Study [56]. In a study by Motivala et al. [57], a study on chronic insomnia confirmed a decrease in nocturnal ghrelin, but leptin did not show a significant difference in the control group. Based on these results, insomnia is thought to induce inappropriate overeating and weight gain. Melatonin, a hormone that controls circadian rhythm, is also closely related to insomnia. Various studies have confirmed that melatonin levels are decreased in patients with insomnia. In particular, the longer the insomnia period, the greater the decrease in melatonin [58].

The immune system is also reciprocally related to sleep, and sleep regulation is regulated by the immune system. It is also established that sleep deprivation affects the immune system [59,60]. During sleep, cytokines such as interleukin 1 and tumor necrosis factor are known to be involved in non-rapid eye movement (NREM) sleep. Sleep deprivation is known to cause changes in immune cell number, immune function, and cytokines, as well as to make them more susceptible to viral and bacterial infections [61]. In addition, chronic insomnia has been reported to increase inflammation and mortality rates [62].

Insomnia induces various neurophysiological changes. In a meta-analysis that analyzed 23 studies using polysomnography in insomnia patients, it confirmed the changes in various sleep variables, including sleep efficiency index, total sleep time, rate of slow-wave sleep, rate of rapid eye movement (REM) sleep, and rate of...
change in the wake time [63]. In addition, these neurophysiological changes were observed during the daytime. High-beta power was observed in the resting state EEG of an insomnia patient, and a change in the P200 component was reported in the event-related potential study [64,65]. These daytime neurophysiological changes are known to reflect the hyperarousal state caused by insomnia.

**Insomnia and physical disorders**

Insomnia is known to cause temporary changes in various physiological functions as well as various physical diseases [66]. Since insomnia is accompanied by changes in heart rate and blood pressure, several studies have been conducted in the past to determine whether insomnia is related to cardiovascular diseases such as hypertension, coronary heart disease, and heart failure; however, each study revealed inconsistent results [67]. However, several meta-analysis studies and nationwide population-based studies conducted over the past decade have reported that insomnia increases the risk of cardiovascular disease [67-69]. Sofi et al. [70] concluded in a meta-analysis that insomnia was associated with the development of cardiovascular disease and cardiovascular disease mortality. The onset of cardiovascular disease is known to be associated with dysregulation of the autonomic nervous system due to insomnia and metabolic syndrome due to hormonal dysregulation [71].

Insomnia is also known to cause endocrine disorders. In the case of type 2 diabetes mellitus, several studies have slightly different results, but several studies have suggested that insomnia and type 2 diabetes mellitus are related, and that insomnia may act as a risk factor for type 2 diabetes mellitus [72,73]. Although it is well-known that abnormal thyroid function induces insomnia, the effect of insomnia on thyroid function has not been well studied [66]. However, elevated levels of thyrotropin-releasing hormone and thyroid-stimulating hormone have been reported in patients with insomnia accompanied by depression [74]. Obesity has also been reported to increase the risk of insomnia, and changes in appetite-regulating hormones, such as leptin and ghrelin, are known to be involved. However, a recently reported meta-analysis confirmed that insomnia did not directly increase the risk of obesity [75,76]. Therefore, further evaluation is needed to determine whether insomnia increases the risk of obesity.

Insomnia is known to increase the risk of infection and cancer because of the close relationship between sleep and the immune system [38]. A cohort study confirmed that insomnia increases the risk of respiratory tract infection, and it has been reported that patients receiving chemotherapy are more susceptible to infection if they complain of insomnia [77,78]. Many researchers have long believed that insomnia increases the risk of cancer [79]. This is not just a belief; some studies have reported that insomnia increases the risk of breast cancer [80,81]. In addition, according to a meta-analysis of cohort studies on insomnia, insomnia increases the risk of all cancers overall, but only thyroid cancer reported a significant increase in risk due to insomnia in specific cancer types [82].

**Insomnia as systemic disease**

We looked at examples in previous chapters where insomnia affects various systems of the body as well as the neuropsychiatric domain. Insomnia is characterized as a systemic disease as the role of sleep is not only limited to rest and conservation of energy. Moreover, sleep has an active, complex, and unique function in the body. Sleep can be divided into REM and NREM stages, and it is known that the two stages play different roles [83]. NREM stage is known as the most restorative stage, and it is known to play an important role in energy conservation and maintenance of physical health, such as hormonal regulation, β-amyloid clearance, and adaptive immune response [84]. REM sleep is a stage clearly distinguished from NREM sleep in neurophysiological characteristics, and it is known that eye movement, voluntary muscle paralysis and dreaming appear [83]. REM sleep is related to the process of emotional memory. Also, in REM sleep, muscle tone is actively suppressed, and autonomic and respiratory activation appear [85].

As such, sleep is a very dynamic process in which stages have different purposes and appear alternately, and each stage has a bidirectional relationship with different biological systems. Therefore, if this complex process is disturbed due to insomnia, it inevitably has a systemic effect on the body and mental health.

Furthermore, insomnia has a systemic effect and is not limited to a specific area as it affects distributed systems throughout the body. An example is a decrease in immune function due to insomnia. Chronic insomnia increases inflammation in the body, making it vulnerable to infection. The hyperarousal model, which is one of the main causes of insomnia, also affects the autonomic nervous system distributed in all parts of the body, so it affects not only the central nervous system but also the peripheral nervous system and various organs throughout the body. In addition, the endocrine and circulatory systems are also greatly affected.

Moreover, the systemic effect of insomnia is not limited to the body but is known to directly or indirectly affect social and occupational functions [86,87]. The array of systemic effects and comorbidities caused by insomnia is known to be directly related to a reduced quality of life for patients across a range of different domains [88]. Although the treatment of insomnia is important due to its
systemic effect, it has been reported that many subjects with insomnia symptoms do not feel the need for treatment or do not receive appropriate treatment [89]. The rationale many patients neglect insomnia even though they are symptomatic is because its negative effects are undermined and its systemic sequelae are not well-known [90]. Therefore, we expect that this review, which studied the characteristics of insomnia as a systemic disease, will help reduce the enormous personal and social burden of untreated insomnia and prevent insomnia-induced secondary diseases.

**Conclusion**

Insomnia not only causes problems related to sleep initiation and maintenance but also has a secondary global sequela on both the mind and body. Insomnia causes functional impairment in various cognitive domains and causes emotional dysregulation. Chronic insomnia also increases the risk of various diseases, such as major depressive disorder and Alzheimer dementia. However, insomnia is not limited to these neuropsychiatric areas, but it affects various physiological systems such as the immune system, endocrine system, and circulatory system and increases the risk of various diseases such as diabetes mellitus, hypertension, cancer, and infection. Insomnia is considered a systemic disease because the sleep process is a very complex, dynamic, and globally biological phenomenon. However, despite the negative effects of insomnia, the proportion of patients who do not receive optimal treatment is very high. This is due to the very limited view of the negative effects of insomnia on our bodies. Therefore, recognizing insomnia as a systemic disease is expected to help prevent secondary impairment and reduce costs through early detection and appropriate treatment.

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Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by abnormalities in social communication/interaction and restrictive, repetitive patterns of behavior. ASD is a relatively common psychiatric disorder, with a prevalence of approximately 1.7% in children. Although many children and adolescents with ASD visit the hospital for medical help for emotional and behavioral problems such as mood instability and self-harming behavior, there are also many visits for sleep disturbances such as insomnia and sleep resistance. Sleep disturbances are likely to increase fatigue and daytime sleepiness, impaired concentration, negatively impact on daytime functioning, and pose challenges in controlling anger and aggressive behavior. Sleep disturbance in children and adolescents with ASD negatively affects the quality of life, nothing to say the quality of life of their families and school members. In this review, sleep disturbances that are common in children and adolescents with ASD and adolescents are presented. The developmental and behavioral impacts of sleep disturbances in ASD were also considered. Finally, non-pharmacological and pharmacological treatments for sleep disturbances in children and adolescents with ASD and adolescents are reviewed.

**Keywords:** Autism spectrum disorder; Insomnia; Melatonin; Problem behavior; Sleep

**Introduction**

Autism spectrum disorder (ASD) is a common neurodevelopmental disorder with a prevalence of approximately 1.7% in the general population. ASD is a disease with abnormalities in two domains: social communication/interaction and restrictive, repetitive patterns of behavior [1]. ASD is a multi-genetic disorder with a strong genetic tendency, occurring about four times more in men than in women, which is postulated to be related to a female protective effect [2].

Many ASD symptoms begin early in life and persist throughout life. Typical ASD symptoms include internalizing problems such as anxiety, depression, somatization, and externalizing problems such as rule-breaking, aggression, self-harm, hyperactivity, impulsivity, and core deficits. These ASD symptoms have a significant negative impact on the quality of life, and that of their families and school members. Therefore, ASD has a significant impact on public health and education systems [3].

Sleep is an important physiological phenomenon for typical synaptic development and brain maturation, and children with sleep disturbances are vulnerable to emotional and cognitive development [4]. In particular, rapid eye movement sleep disturbance is associated with neural maturation and organization difficulties in children with ASD [5]. Sleep disturbances of children and adolescents with ASD are necessary to recognize and treat actively as they persist for a long time, and can change the sleeping patterns of...
parents and siblings sharing the same living space [6].

The causes, impact on daytime activity, evaluation, and treatment of sleep disturbance in children and adolescents with ASD were reviewed.

**Are sleep disturbances common in children and adolescents with autism spectrum disorder?**

Sleep disturbance in children and adolescents with ASD is the most agonizing symptom for parents and one of the primary reasons for seeking psychiatric assistance [7]. Commonly, the prevalence of sleep disturbance in children and adolescents with ASD is approximately 60% to 86%, which is 2 to 3 times that of typically developed children [8]. Their sleep disturbances are not related to the level of cognitive and language development.

There are many different types of sleep disturbances. Difficulty in sleep onset and maintenance, decreased total sleep time (TST), frequent night waking, bedtime resistance, excessive daytime sleepiness, and behavioral insomnia in childhood are common [9-11]. In studies using actigraphy and polysomnography, children with ASD reported shorter sleep time, longer sleep latency (SL), and decreased sleep efficiency (SE) compared with typically developed children [12].

**Does sleep disturbance affect behavior and emotional regulation, and daytime functioning in children and adolescents with autism spectrum disorder?**

There is growing interest in the relationship between sleep disturbance and daytime problem behavior in children and adolescents with ASD [9,10]. Sleep disturbance in children and adolescents with ASD negatively affects daytime functioning, including behavioral control, learning function and memory, and leads to increased stress levels in their family members [9,13]. Poor sleep has been linked to increase internalizing and externalizing problems, including tantrums, oppositional behavior, physical aggression, irritability, self-injury, depression, anxiety, mood variability, inattention, and hyperactivity [9,14-16].

The effect on internalizing and externalizing problems varies depending on the degree and type of sleep disturbances. Adams et al. [16] reported that externalizing and overall problem behaviors were highly correlated with the severity of sleep problems, but not with internalizing behaviors. Meanwhile, Sikora et al. [15] suggested that both severe internalizing and externalizing problems were related only to moderate to severe sleep disturbance. In a study of 40 male children with ASD (aged 5–12 years), there was a positive relationship between the severity of ASD symptoms and problem behavior in patients with no or mild sleep disturbance, while there was no significant relationship between the severity of ASD symptoms and problem behavior in patients with moderate-to-severe sleep disturbance [17]. In a polysomnography study of 21 children with ASD, Malow et al. [18] reported that poor sleepers had more affective and social problems, and Goldman et al. [19] reported that sleep fragmentation was correlated with restricted and repetitive behavior. In addition, aggression, self-injury, and tantrum can be predicted when there is a night-to-night variation in sleep onset time and sleep duration [20].

However, the effect of the type or severity of sleep disturbances on the severity of core symptoms, externalization, and internalization symptoms of ASD is still unclear, and further studies are needed to address this issue.

**Causes of sleep disturbance in children and adolescents with autism spectrum disorder**

The causes of sleep disturbance in children and adolescents with ASD have not been clearly identified. Sleep disturbance is correlated with biological, psychological, and social/environmental factors. Abnormalities in melatonin synthesis, hypersensitivity to environmental stimuli, behavioral insomnia in childhood, delayed sleep phase syndrome, rapid eye movement sleep behavior disorder, and accompanying neurological and psychiatric disorders such as anxiety, depression, and epilepsy are considered as the main causes. Additionally, neurophysiological and neurochemical abnormalities, abnormal timing of melatonin secretion, comorbid medical conditions and current medications, core ASD symptoms, child-rearing practices, and family stress have been suggested as causes of sleep disturbance [13]. Sleep disturbance becomes apparent by the underlying biological and behavioral rhythm problems, which are triggered by internal and external stress [21-23].

In this section, environmental and behavioral problems, arousal and sensory dysregulation, circadian-relevant gene abnormalities, melatonin rhythm, peak and receptor site abnormalities, and comorbid psychiatric disorders, which are the main causes of sleep disturbance in children and adolescents with ASD, are reviewed.

1. **Environmental and behavior problems**

   Learned behaviors are suggested to be one of the most common causes of delayed sleep onset and night waking. Many children and adolescents with ASD have a delayed sleep onset and difficulty falling asleep due to increased screen time in video games, mobile
games, and others [24]. In a study on the association between bedroom media access and sleep disturbance in 49 pediatric patients with ASD, it was reported that media-related variables were associated with sleep disturbance in children with ASD [25]. Sleep in children has a bidirectional relationship with parent sleep. It is important to evaluate the parents’ sleep problems, mental health, and parental stress together when evaluating a child’s sleep disturbance [26]. Parents who are vulnerable to stress, and anxious or depressed parents are more likely to create a negative environment for children’s sleep [27]. Maternal autism traits and anxiety, lower family income, and lower paternal education were also related to their sleep disturbances [26]. Lower family income creates a negative socioeconomic environment for children with ASD, exposing them to more disruptive sleep conditions, which can worsen sleep quality.

2. Arousal and sensory dysregulation in insomnia
There is increasing evidence that sleep disturbance in children and adolescents with ASD is related to arousal dysregulation and sensory hyper-reactivity and that a calming strategy is needed to improve their sleep. Increased cognitive activity, such as having considerable thoughts and worries in bed, makes it difficult to initiate sleep [28]. Physiological arousal is also associated with insomnia. Patients with insomnia have high levels of sympathetic activity, which results in high levels of heart rate, body temperature, and heart rate variability, and is related to high levels of norepinephrine (NE) activity [29,30].

The hyper-arousal response in some children with ASD is related to dysfunction of the locus coeruleus (LC)-NE system. When LC is in phasic mode, individuals generally respond to sensory stimuli and perform appropriate tasks through focused attention. However, when the NE release state in the LC becomes a high tonic state, it cannot respond appropriately to the stimulus and becomes a hyper-arousal state [31]. Children with ASD in this state have inattentiveness, impulsivity, hyperactivity, anxiety, panic, and sleep disturbance. Additionally, children with ASD experience a “hyper-arousal state” associated with abnormal interactions between the two systems, sympathetic hyper-arousal or parasympathetic hypo-arousal, which is associated with anxiety, fear, worry, and insomnia due to sensitivity to environmental stimuli [21].

Hypersensitivity to certain sensory stimuli is also associated with sleep disorders in children and adolescents with ASD. In a study of 27 children aged 6 to 12 years with ASD and 27 typically developed children, children with ASD had more atypical sensory behaviors and sleep disturbances than typically developed children [32]. Children with ASD overreact to certain stimuli as they have a low sensory threshold, and this is related to difficulty initiating or maintaining sleep [33].

3. Circadian relevant gene abnormality
Some researchers suggest that clock genes such as human PER1 (hPER1), hPER2, and hPER3, and clock gene-gene interaction may affect not only human sleep but also human social communication and brain development [34]. Genes related to synaptic homeostasis are involved in synaptic development and pruning, as well as mechanisms involved in sleep-wake control [35]. A recent study reported that acetylserotonin O-methyltransferase and variation in cytochrome P450 1A2 in ASD are related to sleep disturbance [36].

4. Melatonin rhythm, peak, and receptor site abnormalities
Melatonin is synthesized in the pineal gland and regulated by MTNR1A and MTNR1B receptors in the suprachiasmatic nucleus and is involved in the initiation and maintenance of sleep, regulation of seasonal cycles, and immune function [37]. Melatonin is closely related to circadian rhythm, and circadian misalignment is related to abnormal melatonin synthesis, altered melatonin secretion patterns, circadian clock gene anomalies [38].

Insomnia in children with ASD is suggested to be related to abnormal melatonin levels [39]. Abnormal production, increased breakdown, and receptor site abnormalities of melatonin induce an increase in SL and night wakening. Patients with ASD have low levels of melatonin and low urine, serum, or plasma levels of melatonin metabolite, urinary 6-sulfatoxymelatonin [22,40,41]. In ASD children with sleep disturbance, low melatonin levels appear to be related to mutations in genes related to the melatonin synthesis pathway.

5. Comorbid psychiatric disorders
Children with ASD are more likely to have attention deficit hyperactivity disorder (ADHD) and anxiety disorders, which are considered high-arousal disorders [42,43]. In a study comparing the mean electrodermal activity (EDA) and electrodermal reaction frequency of two groups by anxiety level (low and high), the mean EDA for the high anxiety group was significantly lower than that of the control and low anxiety groups, suggesting an abnormality in sympathetic activity in children with ASD [44].

Comorbid medical disorders such as epilepsy, asthma, allergies, gastroesophageal reflux diseases (GERDs), and psychiatric disorders such as anxiety, depression, bipolar disorder, psychosis, and ADHD can negatively affect sleep in children and adolescents with ASD [1,26]. Additionally, intellectual disability, sensory integration deficits, ritualistic or self-injurious behaviors, poor communication skills, and limited responsiveness to social cues can interfere
with sleep training and can exacerbate sleep disturbance.

**Evaluation of sleep disturbance**

The evaluation of children and adolescents with ASD with sleep disturbance should include sleep-related history taking, evaluation of sleep environment, and comorbidities. Sleep environment evaluation includes household noise, noise between floors, parental working hours, bedtime routines, and history of comorbid medical conditions that might disrupt sleep, such as GERD, seizures, asthma, allergies, eczema, or enuresis [1].

Sleep questionnaires are often used to evaluate sleep disturbances in children with ASD. They can evaluate sleep disturbance relatively objectively, are easy to apply, and do not require much time, money, and expertise [45].

The Children’s Sleep Habits Questionnaire (CSHQ) consists of a three-point Likert scale that allows parents to develop behavioral and physiological sleep disorders in school-age children. CSHQ is a screening tool to measure overall sleep characteristics and disturbances in children, including bedtime resistance, sleep onset delay, sleep anxiety, night waking, parasomnia, sleep-disordered breathing, and daytime sleepiness [46]. It comprises 27 questions in eight domains, sleep resistance (six questions), sleep onset delay (one question), amount of sleep (three questions), sleep anxiety (four questions), wakefulness after sleep onset (three questions), parasomnia (seven questions), sleep breathing disorder (three questions), and daytime sleepiness (eight questions).

The Aberrant Behavior Checklist can evaluate the severity of problem behaviors: irritability, agitation, and crying, lethargy, social withdrawal, stereotypic behavior, hyperactivity, noncompliance, inappropriate speech, and sleep problems in children with developmental delay [47].

Some children with ASD with restless sleep and night waking may require laboratory tests, such as ferritin levels, to evaluate iron stores [48].

**Management and treatment**

**1. Non-pharmacological management**

Environmental and behavioral strategies are needed to control sleep disturbances in children and adolescents with ASD. Parents need to create a bedroom environment and form bedtime routines to calm down their children. Although these environmental and behavioral strategies are not easy to set up, they can positively alter sleep in children with ASD if performed consistently [49]. The sleep tool kit (STK), developed by the Sleep Committee of the Autism Treatment Network, is a tailored behavioral intervention tool for children and adolescents with insomnia [49]. STK recommends three methods: visual scheduling of positive evening behaviors, a supplemental calming module to lower arousal levels, and a faded bedtime protocol to go to bed when sleepy. Supplementary calming modules include breathing techniques, muscle relaxation techniques, yoga, massage, mindfulness exercises, and warm baths, which help to control arousal and anxiety in children with ASD [49]. Considering the developmental characteristics of children with ASD, unmodified and graduated extinction, positive routines, and bedtime fading are more effective in children under 5 years of age; however, cognitive-behavioral therapy (CBT) is more beneficial in older children and adolescents [50].

Parent-based sleep education (PSE) has been introduced and used to solve sleep problems in children with ASD and adolescents. PSE is a four-page pamphlet designed to help children with sleep disturbances and is available at www.autismspeaks.org. PSE includes creating a stable sleep environment, regular sleep habits and lifestyle, educating a child to sleep alone, avoiding naps, and facilitating daytime activities. However, the positive effects of PSE on sleep have not yet been demonstrated. A randomized study of 36 children (aged 2–10 years) with SL of 30 minutes into two groups reported that the pamphlet-based education group (n = 19) did not significantly improve SL compared with the group that did not (n = 17) [14]. In a study using actigraphy and CSHQ on the effect of individual training and group training for 80 children with ASD aged 2 to 10 years, the difference in outcome according to the mode of education was not significant [49].

Weighted blankets are heavy blankets used commercially to reduce anxiety and insomnia, and some parents with ASD prefer to use weighted blankets for their children. The National Autistic Society also notes that some children may benefit from sleep with a weighted blanket. However, weighted blankets did not have a significantly positive effect on sleep in children and adolescents with ASD. In a 10-month randomized, placebo-controlled crossover study of 73 children and adolescents with ASD (aged 5–16 years), the difference in TST and SL between the group using weighted blankets and the group using control blankets was not significant [51].

Sound-to-sleep (STS) mattresses are manufactured in such a way that specific sounds and vibrations are implanted in the mattress. The use of STS mattress technology can reduce bedtime resistance by allowing children to feel their favorite sounds and vibrations in bed. In a study of 45 children with ASD (aged 2.5–12.9 years), sleep duration and sleep efficiency were improved after 2 weeks of STS mattress use, although there was no significant decrease in SL [52].
2. Pharmacological management

To date, no medication has been approved for the treatment of insomnia in children with ASD so far in Korea that is the same as in the United States. When using medications to solve sleep disturbance in children with ASD, it should be initiated with small doses and the side effects of medications should be considered [53]. Some medications used in clinical settings to control sleep disturbance in children with ASD have been introduced.

Melatonin is one of the most commonly used drugs to control sleep problems in children and adolescents with ASD. Melatonin is known to have a positive effect on reducing SL, lowering sleep resistance, and increasing TST. In 125 children and adolescents (aged 2–17.5 years) with ASD who had sleep disturbance lasting more than 3 months and did not affect behavioral therapy for 4 weeks, TST was increased by 57.5 minutes, and SL was decreased by 39.6 minutes when 2 to 5 mg of melatonin mini-tablets were administered for 13 weeks [54]. In a study of 160 children with ASD (aged 4–10 years), 3 mg of melatonin was also helpful in lowering insomnia symptoms and bedtime resistance [55]. In a meta-analysis of 35 studies including five randomized double-blind and placebo-controlled studies, melatonin increase TST by 73 minutes and decrease SL by 66 minutes from baseline [56]. Some children have reported nightmares when using melatonin. However, the side effects of melatonin are uncommon and mild.

Alpha-adrenergic agents (clonidine) and antihistamines (diphenhydramine) are also used to ameliorate prolonged sleep onset and frequent night-waking, although research evidence is weak [57].

Low-dose quetiapine is used to improve sleep disturbance and control problem behaviors such as aggression and irritability in children with ASD. In an 8-week open-label study of 18 patients (aged 13–17 years), quetiapine significantly improved their aggression and sleep disturbance [58]. However, special caution is required as atypical antipsychotics, including quetiapine, may cause restless legs syndrome in cytochrome P450 slow metabolizers.

The H1-receptor antagonist, niaprazine, is a safe drug that has been used in children and adolescents with ASD and insomnia.

Conclusion

Sleep disturbances in children and adolescents with ASD are frequent and can negatively affect their lives and developmental processes, and the life and emotional well-being of their families. Sleep disturbance has a significant negative impact on their daytime functioning and harms the quality of life of children and adolescents with ASD and their families by exacerbating externalizing and internalizing problem behaviors. Therefore, we must recognize sleep disturbances early and manage them actively. Sleep disturbances in children and adolescents with ASD may be caused by environmental problems, sensory hyper-arousal, circadian gene abnormalities, melatonin system abnormalities, and accompanying psychiatric disorders. Detailed history taking is required to evaluate sleep disturbance, and a sleep questionnaire can be used as an adjunct. To treat sleep disturbance in children and adolescents with ASD, non-pharmacological management, such as environmental control and CBT, should be prioritized. Medications should be started only if necessary with low doses, and side effects should be closely monitored. The importance of sleep disturbance in ASD must be recognized and the quality of life of patients and their families must be ensured through early and active interventions. Additionally, ongoing research on the causes of sleep disturbance and effective treatment methods is anticipated to continue in children and adolescents with ASD.

Notes

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

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Effects and mechanisms of a mindfulness-based intervention on insomnia

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Introduction

Insomnia is often treated with medication. While such treatments provide only a temporary improvement in sleep disorders for some people, the benefits after drug discontinuation are often diminished, with the negative effects on daytime functioning and risk of addiction [1-3]. One of the common nonpharmacological interventions for insomnia is poor sleep hygiene education, which targets changes in daily behavior and environmental factors that cause sleep deprivation. Among standard clinical treatments, cognitive behavioral therapy (CBT) focuses on regulating sleep needs and modifying sleep expectations, attitudes, and beliefs [1,4]. Since the report of a randomized controlled trial (RCT) published in the Journal of the American Medical Association (JAMA) in 2015 revealed the effects of mindfulness-based interventions (MBIs) on insomnia, a nonpharmacological approach has been expected for mindfulness-based treatments for insomnia [5].

Medication alone is not sufficient to treat insomnia. In addition, the side effects of sleep medications themselves cannot be ignored during treatment. Insomnia begins with poor sleep quality and discomfort, but as it continues, patients fall into a vicious circle of insomnia with negative thoughts and dysfunctional and distorted perceptions related to sleep. Mindfulness-based intervention for insomnia corrects these sequential cognitive and behavioral processes. The mindfulness technique basically recognizes all the thoughts, feelings, and experiences that occur to us as they are, nonjudgmentally, and then trains them to return to the senses of our body. In this way, while noticing all the processes of the sequential vicious cycle and training them to return to our bodies (e.g., breathing), mindfulness determines whether we are really sleepy or just fatigued. This mindfulness-based intervention can be a useful nonpharmaceutical intervention for insomnia, and its stability and efficacy has been proven by many studies.

Keywords: Insomnia; Meditation; Mindfulness; Sleep; Sleep wake disorder
been made to reveal the mechanism of the effect of MBI in a neurobiological way and to investigate this using neuroimaging techniques [15,16]. In recent years, there have been reports that MBI affects the activity of telomerase as well as the improvement of immunological function [7,17,18].

This paper reviewed the effect of MBI on insomnia and also evaluated the mechanistic evidence to aid in future clinical application.

**Vicious cycle of insomnia**

Patients with chronic insomnia describe their condition as a “vicious cycle.” In other words, insomnia worsens as the effort and desire to sleep increases. A strong desire to get more sleep and avoid daytime fatigue leads to a state of feeling trapped between the states of drowsiness and awakening [19]. Several models have been proposed to explain the pathophysiology of insomnia. Among them, Shallcross and Visvanathan [18] presented a model of insomnia that integrated core cognitive and behavioral processes from several extant theoretical frameworks. Insomnia is generally initiated and persisted by the following continuous cognitive and behavioral processes: (1) excessive daytime and nighttime rumination (i.e., excessive daytime and nighttime anxiety and rumination of sleep); (2) primary arousal (i.e., physiological arousal occurs with the first negative assessment of daytime problems caused by sleep deprivation); (3) secondary arousal (i.e., negative secondary or metacognitive judgment of initial arousal and subsequent continuation of physiological arousal); (4) excessive monitoring and selective attention to internal and/or external sleep cues that are consistent or inconsistent with sleep onset; (5) dysfunctional perceived need for control and engagement in sleep effort (i.e., actively taking a nap or increasing sleep opportunities); and (6) distorted perceptions about sleep impairment (i.e., perceptions about sleep impairment as excessively serious). Practical examples for each of these sequential cognitive and behavioral processes would be the following. (1) Rumination: “All day long, there are useless thoughts of sleep in my head.” (2) Primary arousal: “What if I have a problem with my work tomorrow?” (3) Secondary arousal: “Why am I doing this? I feel bad and irritable.” (4) Selective attention/sleep monitoring: “How did I fall asleep normally? I keep observing the feeling of falling asleep.” (5) Dysfunctional perceived need for sleep: “I have to take a nap tomorrow, otherwise I can’t work.” (6) Distorted perceptions: “I’m desperate. If my sleep time is short, I am going to get tired and ruin my work all day tomorrow. What if I get fired?” Misunderstandings of sleep deprivation often lead to excessive negative perceptions of sleep, thus strengthening the vicious cycle of chronic insomnia.

CBT for insomnia (CBT-I) is one of the most widely studied nonpharmacological therapies for insomnia [20]. CBT-I has evolved as a multi-component therapeutic approach, combining the following: (1) cognitive strategies, such as thought restructuring to change sleep-related dysfunctional beliefs and attitudes; (2) behavioral techniques, such as sleep restriction and stimulus control, to promote healthy sleep habits. While this is a good way to mediate the pathophysiological mechanisms of insomnia, a significant proportion (19%–26%) of individuals do not benefit from CBT-I, and the average overall improvement among those who do respond is only 50% to 60% [21]. In order to resolve the ironic situation in which the more obsessed with falling asleep, the more sleep will run away, something other than a cognitive approach is necessary.

**Theoretical rationale of mindfulness-based interventions for insomnia**

While the effectiveness of MBI has been proven by many studies, consistent results have not been reported on the mechanism of therapy. In a review article published in 2016 [14], the psychological mechanisms suggested by existing researchers were systematically reviewed and published; however, each researcher had prioritized these differently. In addition, attempts are being made to clarify the mechanism of the effect of MBI neurobiologically and to investigate this by using neuroimaging techniques [15,16].

During mindfulness meditation, there is a physiological change in the “wakeful hypometabolic state” [22]. The activity of the sympathetic nervous system decreases and the activity of the parasympathetic nervous system, which is important for relaxation and rest, increases, but this state is known to be one that is clearly different from the state of simply resting or sleeping qualitatively and quantitatively [23]. In other words, in addition to relaxing the body, it aims to reduce mental activity by relaxing the mind, thereby providing feedback to the body, leading to deeper physical relaxation. This has been proven in studies that measure physiological changes in the body while practicing mindfulness meditation [22,23]. Body relaxation, such as simple muscle relaxation, is associated with the activation of primary and secondary motor regions. The activity of the paralimbic brain regions, which mediate sympathetic nerve stimulation, such as the anterior cingulate and insula, is known to be a change in both relaxation and mindfulness meditation. It is known that certain areas other than these are additionally activated, and many experienced meditators have reported activation of frontolimbic and frontoparietal neural networks [24-29]. More interesting to note, is that larger, intensified activation is observed in the frontoparietal attention networks in experienced
meditators than in novice meditators with little experience. If mindfulness meditation is not different from simple general relaxation, short-term and long-term meditators are expected to relax similarly and not differ in their physiological or neurophysiological characteristics. These physical and mental relaxation states are helpful for mental as well as physical arousal that occurs in the vicious cycle of insomnia.

The psychological mechanisms by which MBI influences insomnia are as follows. First, MBI helps in insomnia by improving metacognitive awareness. One of the important elements of mindfulness is based on the fact that “there is nothing eternal and unchanging” which is central to Buddhist psychology. With mindfulness meditation training, all experiences are observed as they occur and disappear. In the process, we become aware of constantly changing experiences, and thus, we understand that all experiences are temporary [30]. This process has been called reperceiving or decentering [31] and has been described as the development of an observer perspective [32]. In the literature dealing with MBI, metacognitive awareness, a variety of terms refer to the ability to observe one’s own thoughts or feelings as transient events of the mind, rather than being true and accurate in themselves. It was somewhat mixed with decentering, defusion, distancing, and reperceiving [33]. Through the mindfulness training process, it becomes possible to see the content of consciousness, that is, the moment-to-moment experience more clearly and objectively without identifying oneself with one’s thoughts. Thus, it becomes a therapeutic process that introduces a ‘space’ between one’s own ‘perception’ and ‘reaction’ by leaving the individual’s immediate experience from the observer’s point of view for insight and analysis of habitual patterns of emotions and behaviors. Indeed, evidence that mindfulness meditation training influences metacognitive awareness has been reported in several studies [34,35]. Metacognitive awareness helps people with insomnia to become aware of their thoughts and experiences as and when they are trapped in a vicious cycle of insomnia, which breaks the link to the next step. In fact, there are reports that MBI reduces rumination, which is common in patients with insomnia through metacognitive awareness [36].

Attention control is also a major psychological mechanism by which MBI affects insomnia. The development of attention is one of the key elements of mindfulness meditation training. A general guideline for meditation in line with the mindfulness meditation tradition is “Focus on your breathing in and out. Keep your attention without distracting. If you get distracted, quietly turn your attention to your breath and start over [37].” Attention training improves the ability to maintain nonjudgmental awareness of thought patterns, assumptions, and sensory perception. This awareness helps keep your thoughts and emotions away from being overly intense. With mindfulness meditation, we train to sustain attention to your breathing and redirect your attention to our breathing as an anchor whenever our thoughts wander. This attention control is helpful in rumination, arousal, and selective attention/sleep monitoring processes in the vicious cycle of insomnia.

The next psychological mechanism of MBI is the reduction of automatic thoughts and self-referential thinking. Automatic thinking begins unconsciously and is not easy to interfere with or prevent. In other words, when consciousness cannot consciously attract attention, the default mode network (DMN) starts involuntarily [38]. Objective perception of thinking through mindfulness meditation interprets thinking as ‘just thoughts’ and prevents unreasonable negative thinking from seeing as facts. Objective recognition of these automatic thoughts through mindfulness meditation is known as a major mechanism by which MBI reduces depression, anxiety, and stress [39]. Parts of the DMN, especially cortical midline structures (CMS), include the medial prefrontal cortex, the anterior cingulate cortex, and the posterior medial cortices, which are automatic thinking [40,41]. It is known to play an important role in CMS, and it has been reported that decreased activity of CMS is associated with a decrease in automatic thinking. CMS is also involved in self-referential thinking, which is a type of automatic thinking known to be associated with mood and anxiety disorders. MBI weakens nonadaptive habitual self-view by affecting the DMN area, especially CMS [16]. Reducing these negative and automatic thoughts through MBI can reduce negative, but extremely subjective, self-referencing thoughts that occur during insomnia. This helps to reduce primary and secondary arousal in the vicious cycle of insomnia.

In addition, meditation is a training of acceptance. Acceptance is a nonjudgmental process, acknowledging and accepting the experience of the mind and body at the present moment as it is. The automatic reaction of the mind hates all pain and runs blindly. However, this autopilot reaction is more painful. In a way of life that accepts pain and can coexist, acknowledging reality and not Escaping, it becomes active approval, not passive defeat. One way to accept pain is through self-compassion. The concept of self-compassion is related to mindfulness [42]. Self-compassion, defined by Neff [41], consists of three components. The first is self-kindness, which refers to an attitude that allows us to have a kind and understanding attitude to ourselves rather than being harsh and critical when we feel painful or inappropriate. The second is common humanity, which is to perceive one’s own experience as part of a greater human experience than to see it as separating and isolating. The third is mindfulness, which refers to the ability to experience one’s painful emotions and thoughts with a balanced awareness rather
than overidentifying with them. As can be seen from the concept itself, mindfulness and self-compassion are very related, and one study reported the correlation between the total score on the mindfulness scale and the total score on the self-compassion scale [43]. In addition, studies have reported that changes in mindfulness can predict changes in self-compassion, and it has been argued that self-compassion partially mediates the relationship between mindfulness and well-being [44]. This acceptance relieves the pain of secondary arousal and continuous distorted and biased perceptions and thoughts that occur in insomnia. Increased acceptance of difficult thoughts, emotions, and physical sensations allowed them to let go of their desire (and desperate behaviors) to make sleep happen.

**Evidence of mindfulness-based interventions for insomnia**

Many studies have been conducted to verify the effect of MBI on insomnia. Representatively, the RCT published in the 2015 JAMA was randomized into two groups for those with moderate sleep disorder (Pittsburgh Sleep Quality Index [PSQI] > 5) among adult subjects (mean ± standard deviation of age, 66.3 ± 7.4 years). A total of 49 patients were randomly assigned to the MBI or general sleep hygiene training for 6 weeks (2 hours per week), and participants in the MBI group showed significant improvement in PSQI compared to those in the control group. The mean difference between groups was 1.8 (95% confidence interval, 0.6–2.9) and the effect size was 0.89. MBI showed significant improvement in secondary health outcomes of insomnia symptoms, depressive symptoms, fatigue disorder, and fatigue intensity compared to the control group (all \( p < 0.05 \)). Nuclear factor kappa B (NF-κB) concentration decreased significantly with time in both groups (\( p < 0.05 \)). No differences were observed between the groups for anxiety, stress, and NF-κB [5]. Studies that include more objective indicators as variables have also been reported accordingly. In one study, mindfulness-based stress reduction (MBSR) and drug treatment groups were randomly assigned, and the Insomnia Severity Index (ISI), PSQI, sleep diaries, and wrist actigraphy were used as objective indicators. When comparing the results after 8 weeks with the baseline, sleep onset latency in the MBSR group decreased by 8.9 minutes (\( p < 0.05 \)) [45]. In a 2015 study of breast cancer survivors, subjects were randomly assigned to the MBSR group and the usual care (UC) group, and objective variables, including actigraphy, were measured. As a result, after 12 weeks of objective sleep variable, MBSR group had a more significant effect than control group in the following areas; sleep efficiency (78.2% of MBSR group vs. 74.6% of UC group, \( p = 0.04 \)), percent of sleep time (81.0% of MBSR group vs. 77.4% of UC group, \( p = 0.02 \)), and less number of waking bouts (93.5 in MBSR group vs. 118.6 in the UC group, \( p < 0.01 \)) [46].

In a 2014 study, a randomized, partially blinded, and noninferiority trial was conducted to determine whether MBSR was inferior to CBT-I for insomnia. This study was conducted in patients with insomniac cancer, and the evaluation was performed at baseline, immediately after the program, and after 3 months of follow-up. MBSR was inferior to CBT-I immediately after the program but showed noninferiority after follow-up (\( p = 0.02 \)). Although CBT-I is associated with rapid and durable improvement and remains the best option for nonpharmacological treatment of insomnia, MBSR has brought about clinically significant changes in sleep and psychological outcomes [47]. In another three-arm and single-site RCT, 54 subjects were placed in the MBSR group, mindfulness-based therapy for insomnia (MBTI) group, or an 8-week self-monitoring condition. Total wake time (TWT), Pre-Sleep Arousal Scale (PSAS), ISI, and objective sleep measurements were measured using laboratory polysomnography and wrist actigraphy. Subjects who received MBI (MBSR or MBTI) were found to be superior to the self-monitoring control group from baseline to postintervention in TWT (43.75 vs. 1.09), PSAS (7.13 vs. 0.16), and ISI (4.56 vs. 0.06). There was no significant difference between the MBSR and MBTI. From baseline to 6-month follow-up, MBTI showed a greater reduction in ISI score than MBSR (\( p < 0.05 \)), with the largest difference at the 3-month follow-up. Remission and response rates of MBTI and MBSR were maintained until 6 months follow-up, and MBTI showed the highest ratio of treatment remission (50%) and response (78.6%) at 6 months follow-up [48].

As these studies have increased recently, systematic reviews and meta-analysis results of these studies are also reported in literature. In the meta-analysis results of MBI insomnia published in 2020, participants in the MBI group showed a significant improvement in insomnia as measured by the PSQI (\( p < 0.00001 \)) as compared to the control group. In this comprehensive meta-analysis, MBI appears to be effective in treating insomnia. However, the authors suggested that further research is needed to investigate the long-term effects of MBI on insomnia [49].

Each study has used somewhat different techniques for MBI as a basis for insomnia. The MBI that has accumulated the most evidence so far is MBSR, and in the case of mindfulness-based cognitive therapy, subjective improvement is reported, but the most objective evidence is insufficient [50,51]. In the case of MBTI, which is a more specific technique for insomnia, recent studies are underway, and several significant results described above have been reported accordingly.
Mindfulness-based approach to treatment of insomnia

The mindfulness-based approach to insomnia incorporates key elements of MBI. In order to quantitatively report the effect on insomnia, conventional MBIs such as MBSR have been widely used as research results. In recent years, evidence for MBTI specialized in insomnia has also been collected accordingly. If the core elements of mindfulness are properly included, basically, any form of intervention will help with insomnia. However, the therapists will need to be embodied in the MBI; they should have medical knowledge, including that of sleep physiology.

The goal of MBTI is to increase awareness of mental and physical conditions that cause chronic insomnia and to develop adaptive ways for these undesirable conditions [52]. The practice of meditation, discussion, and daily monitoring of sleep and waking activity helps to enhance this awareness. Particular attention is paid to the mental and physical conditions of sleepiness and fatigue, and participants are taught to distinguish between these two conditions. Using awareness as a platform, you are trained to respond to sleep disorders with mindfulness techniques instead of automatically reacting by increasing effort to rest. Participants should avoid meditating to sleep at night. Instead, the meditations are used as a practice of cultivating awareness and mindfulness principles, not to be used as a relaxation strategy for falling asleep. It includes effectively managing emotional responses to sleep disturbance and daytime fatigue as well as reducing unwanted waking at night. As the program progresses, participants are taught to use mindfulness principles and behavioral strategies to work in these undesired conditions. Specific behavioral changes (sleep restriction and stimulus control) are empirically supported techniques for insomnia that complement the mindfulness principle and should be treated together. MBTI consists of three major components: (1) start with activities in the form of formal mindfulness meditation, including one quiet meditation and one movement meditation; (2) discussions are guided by MBTI leaders when participants are asked about their findings during meditation and their application to insomnia; and (3) the didactic period includes education about sleep and wake physiology and instructions for stimulus control and sleep restriction [52].

Conclusion

Insomnia begins with the discomfort of sleep itself, but as it continues, patients fall into a vicious circle with negative thoughts related to sleep. As such, those who experience chronic insomnia fall into a vicious cycle of “increasing sleep worsens as the desire and effort to sleep increases” which in turn leads to a strong desire to get more sleep and avoid daytime fatigue. MBI corrects sequential cognitive and behavioral processes and can be a useful nonpharmaceutical intervention for insomnia. In many studies, MBI for insomnia has been proven with stability and efficacy.

Notes

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

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Avulsion injuries: an update on radiologic findings

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Avulsion injuries result from the application of a tensile force to a musculoskeletal unit or ligament. Although injuries tend to occur more commonly in skeletally immature populations due to the weakness of their apophysis, adults may also be subject to avulsion fractures, particularly those with osteoporotic bones. The most common sites of avulsion injuries in adolescents and children are apophyses of the pelvis and knee. In adults, avulsion injuries commonly occur within the tendon due to underlying degeneration or tendinosis. However, any location can be involved in avulsion injuries. Radiography is the first imaging modality to diagnose avulsion injury, although advanced imaging modalities are occasionally required to identify subtle lesions or to fully delineate the extent of the injury. Ultrasonography has a high spatial resolution with a dynamic assessment potential and allows the comparison of a bone avulsion with the opposite side. Computed tomography is more sensitive for depicting a tiny osseous fragment located adjacent to the expected attachment site of a ligament, tendon, or capsule. Moreover, magnetic resonance imaging is the best imaging modality for the evaluation of soft tissue abnormalities, especially the affected muscles, tendons, and ligaments. Acute avulsion injuries usually manifest as avulsed bone fragments. In contrast, chronic injuries can easily mimic other disease processes, such as infections or neoplasms. Therefore, recognizing the vulnerable sites and characteristic imaging features of avulsion fractures would be helpful in ensuring accurate diagnosis and appropriate patient management. To this end, familiarity with musculoskeletal anatomy and mechanism of injury is necessary.

Keywords: Apophyseal injury; Avulsion fractures; Diagnosis; Cumulative trauma disorders; Athletic injuries; Therapeutics

Introduction

In modern society, sports activities play an important role in the lives of many people. With an increasing number of people participating in dynamic sports activities, avulsion injuries are becoming more prevalent. Skeletally immature athletes are at particular risk for injuries at the apophysis, as it is weaker than the attached muscle-tendon unit. Certain types of avulsion fractures are seen more frequently in elderly or osteoporotic patients [1,2]. There are two distinct types of avulsion injuries: acute and chronic. Acute avulsion injuries result from sudden, forceful, or unbalanced contraction of the musculotendinous unit [1]. In contrast, chronic injuries are due to repetitive submaximal force, which may result in minimal displacement or widening of the physis [1]. In acute injuries, athletes experience sudden, shooting pain, swelling, and local tenderness. A clear history is available in most cases...
of apophyseal injuries. The diagnosis is easily confirmed on the basis of physical findings, symptoms, patient’s age, biomechanical analysis of the injury, and radiography. However, patients with chronic overuse injuries usually present with a gradual onset of pain and have no clear history of trauma. Radiographs may also show normal findings, which confounds the diagnosis. In addition, posttraumatic bone changes may resemble other disease conditions, such as inflammation or neoplasm [3,4]. Therefore, imaging plays an important role in detecting and understanding avulsion fractures. Computed tomography (CT) is helpful for detecting tiny avulsed bone fragments in cases showing inadequate or equivocal radiographic findings [4]. Ultrasonography (US) with a high-frequency transducer has the advantages of no exposure to radiation, early detection even without an ossification center, and the possibility of dynamic examination [5]. However, the usefulness of US is limited in cases showing artifacts or lesions in a deep location. Magnetic resonance imaging (MRI) is the best modality for evaluating edematous changes in bone and soft tissue that help radiologists detect the affected muscles, tendons, ligaments, and associated bony lesions [3,4].

To avoid performing excessive imaging and biopsies or making incorrect diagnoses, anatomical knowledge and understanding of the pathophysiology of avulsion injuries are important. In this review, we summarize and illustrate the musculoskeletal anatomy and the radiographic, US, CT, and MRI features of avulsion injuries in the whole body, including the lower extremity (e.g., pelvis and femur, knee, ankle, and foot) and upper extremity (e.g., shoulder, elbow, and hand) (Fig. 1).

Pelvis and femur avulsion injuries

Acute avulsion injuries of the pelvis and femur are divided into two groups based on the mechanism underlying the injury. The most common injury mechanism is an abrupt, strong concentric or eccentric contraction of a large muscle that occurs during an attempt to accelerate or decelerate the body mass [6-8]. The second mechanism is abrupt immoderate passive lengthening, which can cause ischial avulsion in dancers or cheerleaders and can occur in individuals performing anteroposterior stretching or "the splits" without external force [6,8]. Apophyseal avulsion injuries of the hip and pelvis usually occur in adolescent athletes and can occur in athletes as late as their mid-20s [6]. The apophyseal physis usually fuses later than the physes of long bones [8].

In the largest study evaluating these injuries, Rossi and Dragoni [9] found that the most common locations were the ischial tuberosity (54%), the anterior inferior iliac spine (AIIS) (22%), the anterior superior iliac spine (ASIS) (19%), the superior corner of the pubic symphysis (3%), and the iliac crest (1%). Common avulsion injuries of the femur occur at the greater and lesser trochanters (Fig. 1).

1. Ischial tuberosity avulsion fracture

The ischial tuberosity is the insertion site of the adductor muscle and hamstring muscle group, which is composed of the semimembranosus, biceps femoris, and semitendinosus. A forceful pull on these muscles before the apophysis is closed leads to the avulsion of the open ischial apophysis [8]. An avulsion injury is caused by extreme contraction of the hamstring muscles during high-speed running, for example, during sprinting, or when stretching is performed at an extreme joint position, for example, during water skiing or playing American football [10]. Patients present with extensive buttock pain, ecchymosis, swelling, bruising in the thigh, and decreased hamstring strength. In the acute phase, ischial epiphysiolysis presents as a non-displaced avulsion on a radiograph, which appears as a curved, well-demarcated bone fragment adjacent to its origin [1]. US, CT, and MRI can be useful for diagnosis if radiographs are normal or subtle. MRI is the most accurate imaging tool for diagnosis, especially for identifying non-displaced or minimally displaced avulsion [1,3]. On MRI, acute avulsion injuries appear as focal fluid signal intensity intervening between the tendon edge and the ischial tuberosity (Fig. 2). Fluid collection and soft tissue edema are often observed at the site of injury [3,11]. Chronic avulsion injuries often result in prominent heterotopic bone formation or callus formation, which mimics osteomyelitis or Ewing sarcoma [1,8]. In such cases, CT may be helpful for the diagnosis [12]. Acute avulsion injuries with a displacement of < 15 mm tend to respond well to conservative treatment. Early surgical intervention can be considered for injuries with significant displacement (> 15 mm) [13,14].

2. Anterior superior iliac spine and anterior inferior iliac spine avulsion fractures

Other commonly involved avulsion sites in the pelvis are the ASIS and the AIIS. The ASIS is the attachment site of the sartorius and tensor fascia lata, while the AIIS is the origin of the straight head of the rectus femoris. Avulsion injuries of the ASIS and AIIS are commonly believed to result from forceful extension of the hip and flexion of the knee during sports activities, especially during the kicking phase of soccer or rugby and the starting phase of running or jumping [15,16]. Direct trauma or chronic traction rarely causes avulsion injuries. Most patients report acute pain in the iliac region and sometimes show a palpable bony fragment in these regions. Radiographs usually show avulsion fractures of the apophysis involved. However, radiographic findings for non-ossi-
Fig. 1. The diagram shows the vulnerable sites for avulsion injuries in the body. ASIS, anterior superior iliac spine; AIIS, anterior inferior iliac spine; ACL, anterior cruciate ligament; LCL, lateral collateral ligament. Provided by Inje University Busan Paik Hospital.

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fied apophysis can be occult and confusing due to skeletal immaturity. In this case, CT and US can depict a small bone fragment, and MRI can show edema of the affected bone and muscles and fluid collection around the avulsed site (Figs. 3, 4) [17]. The treatment of avulsion injuries of the iliac spine is usually conservative and has a relatively shorter recovery time compared to injuries of the ischial tuberosity. However, surgical treatment has been advocated for patients with the displacement of the avulsed fragment > 2 cm, continued pain following nonunion, or impingement syndrome [14,18].

3. Iliac crest avulsion fracture

Avulsion injury of the iliac crest is uncommon and results from the sudden contraction of the abdominal muscles, including the external and internal abdominal oblique, transverse abdominis, gluteus medius, and tensor fascia latae muscles [15]. The iliac crest apophysis remains cartilaginous until adolescence; the fusion of the ossified apophysis to the iliac bone begins at approximately 15 years of age [19], increasing the vulnerability to overuse and acute and chronic trauma. Clinical examination reveals pain, point tenderness, and swelling along the iliac crest. Asymmetry and apophyseal widening (> 2 mm) of the iliac crest is a radiographic indication of avulsion injury [19]. Segmentation of the apophysis, which is a developmental variant, should not be mistaken for an avulsion of the apophysis. Comparison with the con-

Fig. 2. Avulsion of the ischial tuberosity in a 16-year-old soccer player with pain for 1 month in the left gluteal region. (A) The anteroposterior radiograph of the pelvis reveals small bone fragments (arrow) around the left ischial tuberosity, which has a cortical irregularity (arrowhead). (B) The coronal T2-weighted magnetic resonance image with fat saturation shows bone marrow edema at the widened apophysis of the left ischial tuberosity (arrow). Provided by Inje University Busan Paik Hospital.

Fig. 3. Avulsion of the anterior superior iliac spine (ASIS) in a 17-year-old soccer player with pain for 2 weeks in the right hip. (A) The anteroposterior radiograph and (B) the three-dimensional volume-rendered image of the pelvis show an avulsion fracture of the ASIS (arrows). The bone fragment is sharply defined and displaced inferiorly. Provided by Inje University Busan Paik Hospital.
The iliac crest avulsion might be helpful in differentiating the segmentation of the apophysis from avulsion. However, iliac crest avulsion is sometimes bilateral, which may cause problems in making an accurate diagnosis. In such cases, MRI may be helpful. MRI demonstrates displacement of the apophysis with fluid-like signal intensity in the disrupted physis of the iliac crest and adjacent soft tissue edema (Fig. 5) [3]. Relatively good clinical outcomes are achieved by conservative treatment alone. Surgical treatment can be performed in cases with a fragment displacement of > 3 cm or persistent symptoms [15,20].

4. Pubic symphysis avulsion fracture
The symphysis pubis and inferior pubic ramus are the origins of the adductor longus, adductor brevis, and gracilis muscles. Injuries in this region are secondary to chronic repetitive microtrauma with excessive twisting and turning movements of the body and commonly occur in those involved in soccer, ice hockey, fencing, and tennis [1,3]. However, it may also occur acutely, such as when two soccer players kick the ball simultaneously [1]. The most sensitive imaging modality for these lesions is MRI because these injuries do not often result in a displaced fragment but rather present as a soft tissue injury [3,15]. There are no specific treatment guidelines for pubic symphysis avulsions. However, conservative
treatment may be a safe option and may be superior to surgical treatment [21,22].

5. Femur avulsion fracture
Avulsion injuries of the greater or lesser trochanter are less common than pelvic avulsion injuries; however, these lesions cause substantial pain and dysfunction. The greater and lesser trochanters are the insertion sites for the hip rotators and iliopsoas muscles, respectively. Avulsion injuries of the greater trochanter may occur following forced external rotation of the leg with contraction of the gluteus medius and gluteus minimus muscles. Fractures of the greater trochanter can lead to osteonecrosis of the femoral head [1,23]. In adults, isolated fractures of the lesser trochanter without a history of trauma are usually secondary to metastatic disease. However, avulsion injuries in children and adolescents may occur in sprinters or jumpers during forceful contraction of the iliopsoas tendon with hip flexion [24,25]. On plain radiography, avulsed bone fragments are seen displaced from their origin in many patients (Fig. 6). A confusing radiograph of patients with an uncertain clinical history sometimes requires an additional MRI to evaluate the muscles, ligaments, and underlying lesions of the femur (e.g., metastatic nodules). Conservative treatment of the lesser trochanter avulsion shows excellent results in most cases [25]. However, there is no consensus on the best treatment for avulsion injury of the greater trochanter [26].

6. Thigh splints
Adductor insertion avulsion syndrome or thigh splints are stress-related avulsion injuries that occur at the insertion of the adductor muscles of the proximal medial diaphysis of the femur. Radiographs may be normal in the early stage but may later show a periosteal reaction along the posteromedial cortex of the femoral shaft and possible stress fracture [27,28]. MRI of these lesions presents with periosteal edema and abnormal signal intensity in the medullary cavity or cortex (Fig. 7). These findings can easily be misinterpreted as osteomyelitis or malignant neoplasms, such as Ewing’s sarcoma or osteosarcoma. The absence of bone destruction and soft tissue mass helps to rule out these conditions [28,29]. In addition, the clinical history, location, and symptoms are important in differentiating avulsion injuries from other pathologic conditions. These injuries usually respond relatively quickly to rest for 1–2 months [28].

Knee avulsion injuries
The knee joint is another common site of avulsion injury. The knee joint is a complex joint composed of numerous tendinous, ligamentous, and meniscal attachments that are vulnerable to trauma. A variety of avulsion fractures of the knee can occur, including Segond and reverse Segond fractures, avulsions of the anterior and posterior cruciate ligaments, arcuate complex avulsion, iliotibial band avulsion, avulsions of the biceps femoris, semimembranosus, and quadriceps tendons, Sinding-Larsen-Johansson syndrome, and Osgood-Schlatter disease [30]. Complex knee injuries result from multiple forces such as varus force, valgus force, hyperextension, hyperflexion, internal rotation, external rotation, anterior or posterior translation, and axial load [31].

1. Segond fracture
Segond fracture was classically described as a cortical avulsion of the lateral capsular ligament at the anterolateral proximal tibia. This injury occurs following excessive tension to the lateral capsular ligament due to varus stress on the partially flexed and internally rotated knee [32,33]. Previous descriptions of this ligament include different nomenclatures such as the lateral capsular ligament [32,33], the anterior oblique band of the fibular collateral ligament [34], and the capsule-osseous layer of the iliotibial tract [35]. Recent studies described this structure as a distinct ligament, called “anterolateral ligament” [36,37], but there is no consensus in the terminology of the anterolateral capsular structure. This injury is commonly associated with anterior cruciate ligament (ACL) tears in the adult population [34,38]. However, in skeletally immature patients, the association between Segond

Fig. 6. Avulsion of the greater trochanter in a 53-year-old woman with pain in the right hip for 1 day after a car accident. (A) The anteroposterior radiograph of the right hip faintly shows a small bone fragment (arrow) around the greater trochanter. (B) The coronal computed tomography image shows an avulsion fracture of the right greater trochanter (arrow) of the femur. The small bone fragment without displacement is well defined. Provided by Inje University Busan Paik Hospital.
fracture and ACL tear may not be definitive [39]. The mechanism of this injury results from varus stress on the partially flexed and internally rotated knee [30]. These injuries are important because they may be the only radiologic clue to more serious underlying ligamentous injuries, which vary from an ACL tear to avulsion of the fibular collateral ligament or tibiofibular joint sprains [37,38]. The clinical manifestation of this injury is pain at the lateral joint line with anterolateral instability. The radiograph shows an elliptic bone fragment parallel to the tibia, just below the lateral tibial plateau. In equivocal radiographs, CT more easily shows bone fragments. MRI is generally not required but should be performed to evaluate the associated ligaments and meniscal tears (Fig. 8) [38,40]. The treatment of this injury is based on the severity of the associated ACL and meniscal injuries.

2. Anterior cruciate ligament avulsion fracture

Tibial eminence is the insertion site of the ACL. Although most ACL injuries involve the mid-substance of the ligament, avulsion injury at the tibial insertion site of the ACL rarely occurs and is more common in children than in adults [41]. The clinical manifestations of this injury are pain, flexed knee, and signs of anterior instability [42]. In the pediatric population, this injury commonly occurs with forced flexion of the knee and internal rotation of the tibia. On the other hand, in the adult population, this injury results from forced knee extensions that commonly occur in motor vehicle collisions. This injury in the adult population is more frequently associated with medial collateral liga-

Fig. 7. Adductor insertion avulsion syndrome in a 19-year-old ballet dancer with pain in the right thigh for 3 weeks. (A) The anteroposterior radiograph and (B) the axial computed tomography image of the femur show periosteal reaction (arrow) at the proximal medial diaphysis of the femur. (C) The coronal T2-weighted magnetic resonance image with fat saturation shows bone marrow edema (arrow) and periosteal reaction (arrowhead) at the medial diaphysis of the proximal femur, which is the attachment site for the adductor longus. Provided by Inje University Busan Paik Hospital.

Fig. 8. A Segond fracture in a 67-year-old woman who sustained a rotational injury to the knee. (A) The anteroposterior radiograph of the knee shows an elliptic bone fragment (arrow) arising from the lateral tibial plateau. (B) The coronal T2-weighted magnetic resonance image with fat saturation shows a tiny bone fragment (arrow), which represents an avulsion of the lateral capsular ligament. Marrow edema along the lateral tibial rim (arrowhead) is also present. Provided by Inje University Busan Paik Hospital.
fragments. Moreover, MRI is useful for assessing associated injuries and ACL status (Fig. 9). Injuries with minimally displaced bone fragments are treated conservatively, whereas injuries with markedly displaced bone fragments require surgical treatment (i.e., internal fixation) [44].

3. Arcuate complex avulsion fracture
The arcuate complex and posterolateral aspects of the knee have recently gained attention owing to their complex anatomy and clinical relevance. The arcuate complex primarily consists of the fabellolateral ligament, popliteofibular ligament (i.e., the fibular origin of the popliteus muscle), and the arcuate ligament [33]. The mechanisms of this injury involve forceful direct trauma to the anteromedial tibia with knee extension and varus force to the externally rotated tibia [45]. Clinical manifestations (e.g., swelling and posterior subluxation of the tibia) of this injury are subtle. This makes determining a diagnosis difficult. On plain radiographs, the avulsed bone fragment has a characteristic elliptical appearance arising from the fibular styloid process (i.e., the attachment site for the arcuate complex) and horizontal orientation of its long axis on the anteroposterior radiograph of the knee. This is called the “arcuate sign”. The avulsion may not be conspicuous on a lateral radiograph because of its superimposition on the cortex of the posterior tibial plateau [46]. On MRI, the origin of the bone fragment and associated edema at the fibular head and contiguous soft tissue can be helpful signs for diagnosing this injury (Fig. 10) [46,47].

Arcuate complex injuries are commonly associated with damage to other stabilizing structures of the knee and peroneal nerve. Other conditions encountered in the differential diagnosis of these injuries include injury of the lateral collateral ligament and tendon of the long head of the biceps femoris muscles that are attached to the lateral margin of the fibular head [30,45]. In patients with acute injuries, the primary repair is usually performed by repairing all injured structures [33].

4. Iliotibial band avulsion fracture
The iliotibial band is a complex composed of the tendon of the tensor fascia lata and the deep and superficial fibers of the fascia lata that provide anterolateral stabilization of the knee. The superficial layer is the chief tendinous component that inserts into the Gerdy’s tubercle on the anterior lateral tibia, and the deep layer inserts into the intermuscular septum of the distal femur [33]. This injury only results from a varus force, which is a rare injury mechanism. Isolated tears of the iliotibial band are uncommon [31]. Avulsion fractures of the iliotibial band are usually associated with ACL injuries and patellar dislocations. MRI of this injury shows avulsion with retraction of the iliotibial band from its tibial donor site, the Gerdy’s tubercle, and waviness of the torn fibers (Fig. 11) [48]. A wider injury pattern in patients frequently requires repair of the ruptured iliotibial band [33].

Fig. 9. Avulsion of the tibial eminence in a 45-year-old woman with acute pain and anterior instability of the knee. (A) The oblique radiograph and (B) the coronal computed tomography image of the knee show a bone fragment (arrow) that arises from the attachment site for the anterior cruciate ligament (ACL). (C) The sagittal fast spin-echo proton density-weighted magnetic resonance image with fat saturation reveals a bone fragment avulsed from the tibia with an intact ACL and adjacent marrow edema (arrow). Provided by Inje University Busan Paik Hospital.
5. Patellar tendon avulsion fracture

1) Proximal patellar tendon avulsion fracture
Avulsion injuries of the inferior pole of the patella and the proximal patellar tendon include a wide spectrum that consists of “jumper’s knee,” patellar sleeve avulsion, and Sinding-Larsen-Johansson syndrome. The inferior pole of the patella is the origin of the patellar tendon, which inserts into the tibial tuberosity [30]. These injuries result from excessive quadriceps contraction against resistance, which can occur in basketball, volleyball, and football. “Jumper’s knee” is the thickening of the patellar tendon but without demonstrable avulsion or tear. Patellar sleeve avulsion is an injury to the cartilaginous portion of the inferior pole of the patella, whereas Sinding-Larsen-Johansson syndrome is a pure osseous injury without cartilaginous injury (Fig. 12). On plain radiographs, these injuries appear similar to small bone fragments at the inferior pole of the patella. To differentiate between the two entities, MRI is required that reveals extensive cartilaginous or pure osseous injury at the inferior pole of the patella [49]. Treatments for displaced patellar sleeve avulsion include open reduction and internal fixation with reconstruction of the extensor apparatus, whereas minimally displaced fractures in Sinding-Larsen-Johansson syndrome are usually treated conservatively [49, 50].

Fig. 10. Avulsion of the arcuate complex in a 36-year-old man who had a car accident. (A) The anteroposterior radiograph of the knee shows two small bone fragments (arrows) arising from the fibular head. Another elliptic bone fragment (arrowhead) arises from the medial tibial plateau, which represents an avulsion of the deep capsular component of the medial collateral ligament (reverse Segond fracture). (B) The three-dimensional volume-rendered computed tomography image shows two combined avulsion fractures of the arcuate complex (arrow) and the conjoined tendon of the lateral collateral ligament and the long head of the biceps femoris (arrowhead). The right proximal tibia has a fracture. (C) The coronal T1-weighted magnetic resonance image shows the avulsion of the arcuate complex (arrow) from the styloid process of the fibular head (the arcuate sign). Irregularity of the donor site from the fibular head is also seen (arrowhead). Provided by Inje University Busan Paik Hospital.

Fig. 11. Avulsion of the iliotibial band in a 40-year-old man who had a car accident. The coronal T1-weighted magnetic resonance image shows a bone fragment (white arrow) at the anterolateral aspect of the lateral tibial plateau, which is the attachment site for the iliotibial tract (arrowheads). Irregularity of the donor site from the lateral tibial cortex is also seen (black arrow). Provided by Inje University Busan Paik Hospital.

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2) Distal patellar tendon avulsion fracture
The tibial tubercle is the insertion site of the patellar tendon. Avulsion injuries of the tibial tubercle are infrequent fractures that affect physically active adolescents. Injuries are associated with active extension of the knee and excessive contraction of the quadriceps muscles during jumping sports. Osgood-Schlatter disease may predispose to avulsion fracture of the tibial tubercle [51]. Diagnosis is primarily based on conventional radiography. Oblique radiographs of the proximal tibia and CT are useful for better recognition of the tubercle and fracture pattern [51,52]. MRI is helpful for assessing associated meniscal and ligament injuries.

Osgood-Schlatter disease is a traction apophysitis that results from repetitive microtrauma and traction on the tibial tubercle by the patellar tendon. The entity frequently occurs in active male adolescents who participate in sports that require jumping, squatting, and kicking [30]. On plain radiographs, bony fragments of the tibial tubercle and soft tissue swelling of the anterior aspect of the knee can be detected. Fragmentation of the tibial tubercle alone can mimic normal ossification centers in patients; therefore, MRI is required [53]. MRI can be helpful for confirming this diagnosis, which includes thickening of the infrapatellar tendon, soft-tissue swelling, obliteration of the inferior angle of the infrapatellar fat pad, and bony edema at the proximal tibia contiguous to the tibial tubercle (Fig. 13) [54]. The condition is self-limiting, and complete recovery is usually expected with the closure of the tibial growth plate [54,55].

Ankle and foot avulsion injuries

1. Fifth metatarsal base avulsion fracture
An avulsion fracture of the fifth metatarsal base is a common lower extremity fracture. A forceful pull on the strong structures formed by the short peroneal muscle tendon while applying plantar flexion and ankle inversion on the foot (e.g., unstable landing after jumping or inverted ankle during running) is an important factor in this type of fracture [56]. Some authors have suggested that lateral slip of the plantar fascia is the most important structure involved in this injury [57]. It is important to differentiate this fracture from a Jones fracture, which is a transverse fracture through the junction of the diaphysis and metaphysis without distal extension into the intermetatarsal articulation, and a diaphyseal stress fracture, which occurs within the proximal 1.5 cm of the fifth metadiaphyseal junction because the treatment plan and prognosis for these conditions are different (Fig. 14) [58]. It has been traditionally believed that a Jones fracture and diaphyseal stress may require aggressive treatment. A recent review recommends that an avulsion fracture of the fifth metatarsal base and a Jones fracture should be treated functionally, but diaphyseal stress seems to benefit from early intramedullary screw fixation [59,60].

2. Achilles tendon avulsion fracture
An avulsion fracture of the calcaneal tuberosity is a rare fracture that occurs at the posterosuperior aspect of the calcaneus because of a pull on the Achilles tendon. This injury can occur in patients with osteoporosis and neuropathic disorders (e.g., diabetes mellitus) or can occur due to trauma that causes dorsiflexion of the foot [61]. There are four types of calcaneal avulsion fractures:
type I, simple extra-articular avulsion fracture; type II, “beak” fracture with an oblique fracture line running posterior; type III, infrabursal avulsion fracture involving superficial fibers of the Achilles tendon; type IV, small beak fracture involving the deep fibers of the Achilles tendon [62]. MRI may be helpful for confirmation of type III and IV fractures because it can discriminate between superficial and deep fibers of the tendon. Type I fractures are insufficiency fractures that usually occur as a result of minor trauma such as tripping in elderly patients with osteoporosis. These fractures displace the avulsed fragment superiorly (Fig. 15). However, type III and IV fractures mainly occur in younger patients with severe trauma, such as falling down [62,63]. Treatment options depend on fracture type. Patients with type II fractures are at risk of severe wound complications, such as skin necrosis. Therefore, careful examination of the posterior skin of the heel and urgent reduction with fixation are required if the skin is tented or appears blanched [62].

3. Talar anterior capsular avulsion fracture
Fractures of the talus are generally believed to be relatively uncommon. However, these fractures are the second most common fracture of the tarsal bone. Improved recognition has resulted in an increased number of talar process fractures being diagnosed [64]. Anterior capsular avulsion occurs in basketball players and is a chronic injury caused by microtrauma [1]. A lateral radiograph easily shows a bony protuberance in the concavity of the dorsal talus, corresponding to a cortical avulsion of the broad insertion of the talonavicular joint capsule (Fig. 16).

4. Superior peroneal retinacular avulsion fracture
The superior peroneal retinaculum constitutes the posterolateral border of the peroneal tunnel, thus preventing the displacement of the peroneal tendons [65]. Sudden dorsiflexion of the

Fig. 14. Avulsion fracture of the fifth metatarsal base in a 16-year-old girl with midfoot pain for 1 day. (A) The oblique radiograph of the foot and (B) the three-dimensional volume-rendered computed tomography image show a non-displaced avulsion fracture (arrow) at the tuberosity of the fifth metatarsal base. Provided by Inje University Busan Paik Hospital.

Fig. 15. Avulsion of the calcaneal tuberosity in a 44-year-old woman with diabetes mellitus. (A) The sagittal computed tomography image of the ankle shows an avulsion fracture of the calcaneal tuberosity (arrow), which is the attachment site of the Achilles tendon (arrowheads). The bone fragment is sharply defined and displaced superiorly. The donor site of the calcaneus is also irregular (open arrow). (B) The longitudinal ultrasonography image of the ankle in the prone position depicts a bone fragment (arrow) arising from the calcaneus (asterisk) with mild cephalad displacement. A retracted Achilles tendon (arrowheads) is also seen. Provided by Inje University Busan Paik Hospital.
foot occurring simultaneously with violent contraction of the peroneal muscles can cause a distal fibular avulsion fracture at the attachment of the retinaculum to the lateral malleolus. Radiographs and CT imaging can reveal small linear bony fragments along the lateral margin of the distal fibula. MRI can depict bony fragments and bone marrow edema, as well as subluxation or dislocation of the peroneal tendons (Fig. 17). Nonoperative plaster immobilization is initially attempted; however, surgery may be required for patients with painful, chronic, or unstable dislocations [66].

Shoulder avulsion injuries

1. Greater tuberosity avulsion fracture

Isolated humeral avulsion fractures are rare in the greater tuberosity, which is the attachment site of rotator cuff muscles such as the supraspinatus, infraspinatus, and teres minor tendons. This injury can occur as a result of an extreme pull on the external rotators, after the abduction and external rotation of the arm, after a direct blow to the lateral aspect of the shoulder, after a fall on an outstretched hand with the elbow in full extension or flexion, or after a seizure [67]. The differential diagnosis of this injury is crucial but difficult. Patients with this injury present with decreased abduction strength that mimics rotator cuff injury, which requires prompt surgical repair. However, non-displaced avulsion injuries of the greater tuberosity tend to respond well to conservative treatments, such as immobilization. On plain radiographs, these fractures may be challenging to identify because of osseous overlap. Additional anteroposterior views with internal and external rotation can provide more details regarding a fracture of the greater tuberosity and help identify an occult non-displaced surgical neck fracture [68]. US can be helpful in detecting cortical step-off, which indicates avulsion injury of the greater tuberosity [1]. In patients with clinically suspected rotator cuff injuries, MRI can occasionally show edema at the greater tu-
2. Lesser tuberosity avulsion fracture
Isolated avulsion fracture of the lesser tuberosity is an uncommon injury that may cause significant disability if not diagnosed appropriately. The lesser tuberosity is the attachment site of the subscapularis muscle and tendon. Forceful contraction of the subscapularis muscle to resist abduction and external rotation of the shoulder, and the resultant strong traction force avulses the lesser tuberosity. The most common cause of injury is muscular violence, but a sudden involuntary contraction of the subscapularis muscle during sleep, which may occur during electroconvulsive therapy for psychiatric disorders, may also result in this fracture [69]. An anteroposterior view in maximal internal rotation projecting the lesser tuberosity in the profile usually shows a large displaced fragment. An axillary view is sometimes needed to detect minimally displaced small fragments and is essential to prevent the injury. On plain radiographs, these fractures may be confused with calcific tendonitis of the rotator cuff or osseous Bankart lesions (Fig. 19) [70,71]. CT can properly show even minimally displaced small fragments. Although MRI is not necessary, it allows the evaluation of the entire rotator cuff and better visualization of a minimally displaced fragment [1]. Most acute cases are usually treated by open reduction and internal fixation, but acute non-displaced fractures are treated nonoperatively with satisfactory results. Operative treatment is recommended in patients with a displacement greater than 5 mm or 45° of angulation, blockage of motion, significant clinical weakness, or continued pain [72].

Elbow avulsion injuries
Various mechanisms, such as high compressive-tensile loads, can lead to osseous or chondral avulsion injuries.

1. Little Leaguer’s elbow
The medial epicondyle is the most common site of avulsion injury in the elbow. Before fusion, valgus overload to the medial elbow structures primarily affects the physis, rather than the MCL. Little Leaguer’s elbow results from a chronic overuse injury of the medial epicondyle in skeletally immature throwing athletes (i.e., pitchers) [73]. The initial radiographic evaluation may be normal in up to 85% of the patients. Radiographs may show displacement and fragmentation of the medial epicondyle apophysis. Epicondylar overgrowth resulting from chronic traction injury and adjacent

Fig. 18. An avulsion fracture of the greater tuberosity in a 30-year-old man who experienced shoulder pain after falling. (A) The anteroposterior radiograph of the shoulder shows an avulsion fracture of the greater tuberosity (arrow). (B) The coronal T2-weighted magnetic resonance image with fat saturation reveals marrow edema of the greater tuberosity (arrow). The rotator cuff is intact. Provided by Inje University Busan Paik Hospital.
soft tissue swelling can be observed [74]. MRI can show physeal irregularity or widening between the medial epicondyle and distal humerus and bone marrow edema (Fig. 20). The common flexor tendon may also be thickened [74]. The first line of treatment includes rest from the activity for 4 to 6 weeks and analgesics. Strengthening exercises and physical therapy are also recommended, followed by an interval throwing program [75].

2. Olecranon process avulsion fracture
Avulsion fracture of the olecranon process of the ulna, which is the attachment site of the triceps tendon, is a relatively rare injury, but may be associated with fracture of the radial head [76]. This injury may occur after forceful contraction of the triceps during a fall with an outstretched arm. Radiographs can show a bony fragment that is non-displaced or proximally displaced at the posterior aspect of the proximal ulna. CT can more definitively depict small osseous fragments at the posterior aspect of the proximal ulna. MRI can show a signal change in the triceps tendon and the surrounding soft tissue. Avulsion fractures of the olecranon process should be differentiated from the os supratrochlear dorsale, an accessory ossicle of the elbow. Plate fixation or excision of the fragment with triceps advancement is necessary for most patients. MRI is better for evaluating non-ossified medial epicondylar avulsion fractures [79]. Radiographs and CT imaging show an irregular lucent area, fragmentation or separation of the medial epicondyle, and an apophyseal fragment that can be entrapped in the joint capsule (Fig. 21). In general, less than 1 cm of displacement responds well to conservative treatment, whereas displaced fractures or fractures with an incarcerated fragment are treated by open reduction and screw fixation. Operative options also allow for exploration of ulnar nerve injuries [80,81].

3. Medial epicondyle avulsion fracture
Acute traumatic avulsion fractures of the medial epicondyle occur primarily in boys between the ages of 9 and 14 years; however, it has also been described in adolescents. This injury may occur after a vigorous valgus force of the elbow with simultaneous forearm flexion [78]. Approximately half of medial epicondyle fractures are associated with posterolateral dislocation of the elbow, and often the avulsed fragment becomes trapped within the joint [1,78]. Medial epicondyle avulsion fractures are of two distinct types. Type 1 lesions occur in younger patients and are characterized by a large fragment typically involving the entire epicondyle. Type 2 lesions occur in adolescents and produce small fragments. MRI is better for evaluating non-ossified medial epicondylar avulsion fractures [79]. Radiographs and CT imaging show an irregular lucent area, fragmentation or separation of the medial epicondyle, and an apophyseal fragment that can be entrapped in the joint capsule (Fig. 21). In general, less than 1 cm of displacement responds well to conservative treatment, whereas displaced fractures or fractures with an incarcerated fragment are treated by open reduction and screw fixation. Operative options also allow for exploration of ulnar nerve injuries [80,81].

Hand avulsion injuries
There are several avulsion injuries of the hand and wrist. They have characteristic imaging features and injury mechanisms.

1. Volar plate avulsion fracture
A volar plate avulsion fracture is a common injury that occurs at the proximal interphalangeal joint. The volar plate is a fibrocarti-
laginous structure that is attached to the base of the middle phalanx distal to the joint and prevents hyperextension. Volar plate avulsion fractures typically occur when forceful hyperextension is applied to the fingertip, especially during ball-handling sports [82]. Radiographs show a small fragment of bone avulsed from the volar aspect of the base of the middle phalanx. US can show small bony fragments and adjacent fluid collection at the volar aspect of the base of the middle phalanx. MRI can more definitively depict bone and soft tissue edema and adjacent fluid collection (Fig. 22). Treatment options are conservative and range from early active motion with or without buddy strapping to immobilization at various degrees of flexion/extension [83].

2. Skier’s thumb
Avulsion injury involving the ulnar collateral ligament of the first metacarpal joint is a frequent injury that is called “skier’s thumb” or “gamekeeper’s thumb.” This condition is termed “gamekeeper’s thumb” because the injury is a common chronic occupational injury occurring in British gamekeepers. However, it is more commonly encountered in athletes, especially as an acute injury in skiers; therefore called “skier’s thumb” nowadays [84]. It occurs after violent hyperabduction of the metacarpophalangeal joint. It is more likely to occur if the thumb is simultaneously gripping something. Radiographs may be normal or show a small fragment at the base of the proximal phalanx of thumb; MRI can show abnormally high signal intensity at the base of the proximal phalanx of thumb, which is the distal attachment site of the ulnar collateral ligament (Fig. 23). Nonoperative treatment such as metacarpophalangeal joint immobilization is suggested, but surgical treatment is recommended for a Stener lesion, which occurs when the aponeurosis of the adductor pollicis muscle is interposed between the ruptured ulnar collateral ligament of the thumb and its site of attachment.

Fig. 21. An avulsion fracture of the olecranon in a 64-year-old woman who experienced elbow pain after falling. (A) The lateral radiograph of the elbow and (B) the sagittal computed tomography image show an avulsion fracture of the olecranon (arrow). Provided by Pusan National University Hospital.

Fig. 22. An avulsion fracture of the volar plate in a 20-year-old handball player with acute pain in the second finger after a hyperextension injury. (A) The lateral radiograph of the second finger shows a small bone fragment (arrow) arising from the volar aspect of the base of the middle phalanx. (B) The longitudinal ultrasonography image shows a small bone fragment (arrow) and adjacent fluid collection (arrowhead) at the volar aspect of the base of the middle phalanx. The volar plate is deformed and it has low echogenicity (open arrow). (C) The sagittal T1-weighted magnetic resonance image shows an avulsion fracture of the volar plate (arrow). Irregularity of the donor site from the base of the middle phalanx is also seen (arrowhead). Provided by Inje University Busan Paik Hospital.

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insertion at the base of the proximal phalanx [85].

3. Mallet finger
Mallet finger is a common closed tendon injury that accounts for 2% of all sporting injuries [86]. It occurs when a forceful blow is applied to the tip of the finger, causing a sudden flexion or hyperextension injury. It manifests as an extensor tendon rupture at the insertion site or as an avulsion fracture involving the insertion of the terminal extensor tendon [86,87]. If there is a bony avulsion, radiologic findings of the mallet finger would classically be the presence of a triangular avulsion fragment at the extensor aspect of the distal phalanx at the distal interphalangeal joint (Fig. 24). MRI may show disruption of the extensor tendon in patients without a bony avulsion fragment. The recommended treatment for most mallet finger injuries is the immobilization of the distal interphalangeal joint held in extension by splints. Surgical treatment is only considered in cases of open injuries, failed to splint, palmar subluxation of the distal phalanx, or a large displaced articular fracture fragment greater than one-third of the joint surface [88]. If it is untreated, a swan neck deformity or secondary osteoarthritic changes may develop.

**Conclusion**

Although diverse avulsion injuries occur in all age groups, they primarily affect skeletally immature athletes. These injuries can mimic more serious conditions, such as infection or neoplasm, because of a variety of imaging findings associated with different stages and mechanisms. Conventional radiography is the first imaging modality used to diagnose avulsion injury. In certain cases showing subtle appearances on radiographs, advanced imaging modalities are helpful and can provide additional information to appropriately define the extent of the damage. US has a high spatial resolution with a dynamic assessment potential and allows comparison with the opposite side. CT is more sensitive for depicting a tiny osseous fragment located adjacent to the expected attachment site of a ligament, tendon, or capsule. MRI is the best imaging modality for identifying edematous changes in bone and soft tissue that help radiologists detect the affected muscles, tendons, ligaments, and associated bony lesions. An understanding of the vulnerable sites and pathophysiology of avulsion injuries allows accurate diagnosis and appropriate patient management.

**Notes**

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

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Author contributions
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References
An update on immunotherapy with PD-1 and PD-L1 blockade

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Cancer is the leading cause of death and is on the rise worldwide. Until 2010, the development of targeted treatment was primarily focused on the growth mechanisms of cancer. Since then, drugs with mechanisms related to tumor immunity, especially immune checkpoint inhibitors, have proven effective, and many pharmaceutical companies are striving to develop related drugs. Programmed cell death-1 (PD-1) and programmed cell death ligand-1 (PD-L1) inhibitors have shown great success in various cancer types. They showed durable and sustainable responses and were approved by the U.S. Food and Drug Administration. However, the response to inhibitors showed low percentages of cancer patients; 15% to 20%. Therefore, combination strategies with immunotherapy and conventional treatments were used to overcome the low response rate. Studies on combination therapy have typically reported improvements in the response rate and efficacy in various cancers, including non-small cell lung cancer, small cell lung cancer, breast cancer, and urogenital cancers. The combination of chemotherapy or targeted agents with immunotherapy is one of the leading pathways for cancer treatment.

Keywords: Immunotherapy; Neoplasms; Programmed cell death-1 receptor; Programmed cell death ligand-1 receptor

Introduction

Cancer, the leading cause of death, is on the rise worldwide. According to recently published cancer statistics in Korea in 2018, the growing trend in cancer incidence has slowed compared to 2014, but it can be found that the cancer incidence rate continues to increase until then [1]. Accordingly, there has been a large body of research and progress on gene mutations related to cancer growth and the development of targeted treatments. Until 2010, the development of targeted treatment was mainly focused on cancer’s growth mechanism. However, since then, drugs with mechanisms related to tumor immunity, especially immune checkpoint inhibitors (ICIs), have proven effective [2], and almost all pharmaceutical companies are striving to develop related drugs.

Tumor immunotherapy refers to cancer treatment using the human immune system. Over the past century, cancer researchers have conducted extensive research to treat cancer by strengthening the mechanism by which human immunity recognizes and fights against tumor cells. However, immunotherapy has limitations in its effectiveness and is a structure that strengthens immune-related mechanisms; therefore, it often causes serious side effects, leading to skepticism about tumor immunotherapy among oncologists [3]. However, since 2010, surprising results of tumor immunotherapy, especially monoclonal antibodies related to ICIs, have been reported [4]. Thus, monoclonal antibodies related to ICIs are emerging as a new alternative to metastatic cancer treatment.

Tumor immunotherapy includes not only allogeneic bone marrow transplantation, which has been used for a long time, but it covers various treatment modalities, including tumor vaccines, cytokines, monoclonal antibodies, adoptive cell therapy, and cell
therapy. In this article, we review programmed death-1 (PD-1) and programmed death ligand-1 (PD-L1) inhibitors, which are ICIs that are widely applied in clinical practice.

**Mechanism of PD-1 and PD-L1**

To explain the mechanism of PD-1 and PD-L1, we first discuss the immune cells in the body, especially the T cell lymphocyte immune reaction mechanism. Controlling T cell lymphocyte immune reaction is both complex and precise. For activating T lymphocytes, in advance, the action started with antigen recognition of T cells by binding antigen-bound antigen-presenting cells (APCs) at the T-cell antigen receptor [5]. To fully activate T cells, a stimulatory signal, in which CD80, CD40 expressed on APC surfaces bind the ligand on the T lymphocytes’ surface including CD28, CD40 ligand, was needed. Simultaneously, the inhibitory signal is activated to inactivate the activated T-lymphocyte cells after some time to protect against cell damage resulting from excessive immune reactions [6]. The most recognized inhibitory signals are cytotoxic T-lymphocyte antigen (CTLA)-4, PD-1 expressed on T lymphocytes [7]. In particular, PD-1 is known to regulate the function of T lymphocytes in peripheral tissues by binding ligands of PD-L1 or PD-L2 and PD-1 of T lymphocytes [8]. The immune system of the human body plays a role in the recognition and eradication of mutated tumor cells, but it can also promote tumor growth by selecting tumor cells that can evade immune surveillance [9]. Thus, tumor cells acquire the ability to not eradicate tumor cells by antigen recognition of the immune system.

Moreover, tumor cells that escape the immune system can induce an immunosuppressive status by producing cytokines and growth factors, including vascular endothelial growth factor (VEGF), recruiting T cells, and myeloid-derived suppressor cells [5,9,10]. They can also overexpress the inhibitory ligands on their surfaces to escape the immune system and effectively eradicate tumor cells [11,12]. The inhibitory ligands expressed on tumor cells include PD-L1, as mentioned above. PD-L1 is expressed on the tumor cell surface, while PD-1 is expressed on activated B or T lymphocytes [13]. Thus, binding PD-1 ligands on tumor cells at the PD-1 receptor on lymphocytes prevents the activation of immune cells and maintains the progression without eradication by the immune system, preventing the identification of non-self antigens [14].

PD-L1 is frequently found in various human cancers, including melanoma, lung cancer, renal cell cancer (RCC), head and neck cancer, bladder cancer, ovarian cancer, and gastrointestinal cancer [15]. PD-1 inhibitors or PD-L1 inhibitors prevent the binding of inhibitory signals and sustain the function of eradicating tumor cells by maintaining the activation of T lymphocytes [16]. As a result of studies based on human immune mechanisms on tumor cells, in 2010, a PD-1 inhibitor, pembrolizumab, was developed for melanoma treatment for the first time [2], and the other successful results of various cancers in pembrolizumab and nivolumab led to a new chapter in cancer treatment in current days.

**PD-1 and PD-L1 inhibitors on market: study results and indication**

Currently, most multinational pharmaceutical companies have developed PD-1 or PD-L1 inhibitors for the treatment of various tumors. Here, we describe pembrolizumab, nivolumab, and atezolizumab, which are widely used in Korea.

1. **Nivolumab**

Nivolumab is the first PD-1 inhibitor to be developed to treat refractory metastatic cancers. In December 2014, nivolumab was approved for metastatic or inoperable melanoma by the U.S. Food and Drug Administration (FDA), demonstrating improvement in progression-free survival (PFS) and overall survival (OS) compared to dacarbazine (PFS, 5.1 months vs. 2.2 months; OS, not reached vs. 10.8 months) [17]. Subsequently, showing PFS and OS improvement in the nivolumab group compared to chemotherapy (docetaxel) in metastatic non-small cell lung cancer (NSCLC) second-line treatment [18], nivolumab is gradually expanding and being applied to other cancer types. Currently, its indications are increasing, including malignant melanoma, NSCLC, urothelial cancer, head and neck cancer, hepatocellular cell carcinoma, kidney cancer, Hodgkin’s lymphoma, stomach cancer, and colorectal cancer [19-26] (Table 1).

2. **Pembrolizumab**

Earlier, the pharmaceutical company developed the CTLA-4 inhibitor, ipilimumab, and tested positive results in melanoma [4]. Subsequently, the PD-1 inhibitor pembrolizumab was used in a phase II study in refractory melanoma patients despite using ipilimumab. The study reported statistically significant results that OS in the pembrolizumab group (2 mg/kg) was improved compared to chemotherapy (13.4 months vs. 11 months) and was approved by FDA [27].

In Korea, pembrolizumab was approved for first-line treatment of inoperable or metastatic melanoma, metastatic NSCLC with first-line treatment, and second-line treatment of NSCLC with PD-L1 expression rate above 50% and absence of epidermal growth factor receptor mutation or ALK rearrangement, which progressed to first-line chemotherapy [28,29]. Its indications are
expanding to include Hodgkin’s lymphoma, urothelial cancer, head and neck cancer, and breast cancer [30-34] (Table 2).

3. Atezolizumab
Atezolizumab is a fully-humanized monoclonal antibody against the protein PD-L1, while nivolumab and pembrolizumab are PD-1 inhibitors. Atezolizumab was first approved in advanced urothelial cancer where tumors have progressed after platinum-based chemotherapy by the FDA, showing an improvement in the 12-month OS rate (41%) in a phase 2 study compared to a landmark 12-month OS rate of 20% from an analysis of 10 phase 2 trials who received second-line chemotherapy for advanced urothelial cancer [35] and showed efficacy in platinum-ineligible patients with metastatic urothelial cancer (OS, 15.9 months; PFS, 2.7 months) [36]. Also, atezolizumab was approved by the FDA in palliative second-line treatment of NSCLC showing improvement of OS compared to conventional chemotherapy with docetaxel (OS, 13.8 months vs. 9.5 months; hazard ratio [HR], 0.73; \( p = 0.0003 \)) and metastatic NSCLC which has high expression of PD-L1 first-line treatment by proving the efficacy compared to chemotherapy (OS, 20 months vs. 13.1 months; HR, 0.59; \( p = 0.01 \)) [37,38] (Table 3).

New direction of PD-1 and PD-L1 inhibitors
Although promising results for inhibitors of PD-1 and PD-L1 have been reported, the low response rate of immunotherapy cancer treatment has been reported to be 15% to 20% [39,40]. With the development of immunotherapy leading to PD-1 and PD-L1 inhibitors, efforts to understand tumor immunology last for a better response to immunotherapy. Inflamed tumors that highly infiltrate immune cells and proinflammatory cytokines are known to respond well to immunotherapy. In addition, other immunotherapies, such as CTLA-4 inhibitors, have a better response correlated with posttreatment increases in tumor-infiltrating lymphocytes [41,42]. In other words, an inflamed tumor can have a better response, and immune modulation-induced treatments of weak immunogenic tumors can have similar results, indicating the possibility of therapeutic intervention in immunotherapy. The biomarkers of ICI response have been validated in several studies. Several factors have received much attention, including PD-L1 expression, mutational burden intensity, and deficiencies in antigen presentation [41,43]. PD-L1 expression in tumors was assessed by immunohistochemistry (IHC) staining of PD-L1 positive tumor cells, immune cells, or both cells. High expression PD-L1 in tumors in-

### Table 1. Nivolumab clinical trials results

<table>
<thead>
<tr>
<th>Phase</th>
<th>Population</th>
<th>Therapy</th>
<th>No. of patients</th>
<th>ORR (%)</th>
<th>OS (mo)</th>
<th>PFS (mo)</th>
<th>HR for OS (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III [17]</td>
<td>Metastatic melanoma (BRAF-) / first line</td>
<td>Nivo (210)</td>
<td>50</td>
<td>NR</td>
<td>5.1</td>
<td>0.42 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>III [18]</td>
<td>Metastatic NSCLC (squamous) / second line</td>
<td>Nivo (135)</td>
<td>20</td>
<td>9.2</td>
<td>3.5</td>
<td>0.59 (&lt; 0.001)</td>
<td></td>
</tr>
<tr>
<td>III [19]</td>
<td>Recurrent H&amp;N cancer / second line</td>
<td>Nivo (240)</td>
<td>7.5</td>
<td>2</td>
<td>0.70 (0.01)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ORR, overall response rate; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; BRAF, B-type Raf kinase; Nivo, nivolumab; DTIC, dacarbazine; SLCL, non-small cell lung cancer; H&N, head and neck; GEJ, gastroesophageal junction; MSI-H, microsatellite instability-high; PD-L1, programmed death ligand-1; HCC, hepatocellular carcinoma; RCC, renal cell cancer.

†MSI-H is defined if two or more markers are positive using polymerase chain reaction on tumor tissue.
including melanoma, NSCLC, RCC, prostate cancer, and colorectal cancer showed an objective response to nivolumab using a 5% PD-L1 positivity threshold. However, there is a hurdle that multiple variables of PD-L1 IHC staining caused by transient, intrapatient, and intratumoral heterogeneity and poor uniform test of PD-L1 expression result in poor reliability. In addition, as mentioned above regarding the mechanism of PD-1 inhibitors for cancers, neoantigens produced by somatic mutation of tumor cells as primary drivers of anticancer adaptive immune response have been identified in preclinical data. The long-term clinical benefit of high mutational or neoantigen burden with a mutational load of more than 100 nonsynonymous somatic mutations in cancers has been reported in several studies [44,45].

Some modulators act directly on tumors to increase their immunogenicity, including chemotherapy, radiotherapy, and metabolic modifiers. While conventional chemotherapy has a rapid response initially and has been resistant to tumors in a short time, PD-1 or PD-L1 inhibitors have a lower response rate than conventional chemotherapy and sustained the response in the responding group for long periods. To increase the response rate to immunotherapy, combination treatment with immunotherapy was thought to amplify the antitumor immune response (Fig. 1) [46]. In addition, destroying cancer cells by cytotoxic agents release tumor-associated antigens that can stimulate immune responses to infiltrate the

Table 2. Pembrolizumab (Pem) clinical trials results

<table>
<thead>
<tr>
<th>Phase</th>
<th>Population</th>
<th>Therapy (No. of patients)</th>
<th>ORR (%)</th>
<th>OS (mo)</th>
<th>PFS (mo)</th>
<th>HR for OS (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II [27]</td>
<td>Ipilimumab failed unresectable melanoma</td>
<td>Pem 2 mg/kg (180)</td>
<td>21</td>
<td>13.4</td>
<td>5.4</td>
<td>0.57 (0.001)</td>
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<tr>
<td></td>
<td></td>
<td>Pem 10 mg/kg (181)</td>
<td>25</td>
<td>14.7</td>
<td>5.8</td>
<td>0.50 (0.001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy (179)</td>
<td>4</td>
<td>11</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>III [28]</td>
<td>Metastatic NSCLC / second line</td>
<td>Pem 2 mg/kg (345)</td>
<td>30</td>
<td>10.4</td>
<td>5.0</td>
<td>0.71 (0.0008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pem 10 mg/kg (346)</td>
<td>29</td>
<td>12.7</td>
<td>5.2</td>
<td>0.61 (0.0001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Docetaxel (343)</td>
<td>8</td>
<td>8.5</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td>III [29]</td>
<td>Metastatic NSCLC / first line (PD-L1 ≥ 50%)</td>
<td>Pem (154)</td>
<td>44.8</td>
<td>NR</td>
<td>10.3</td>
<td>0.60 (0.005)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy (151)</td>
<td>27.8</td>
<td>14.5</td>
<td>6.0</td>
<td></td>
</tr>
<tr>
<td>II (single arm) [30]</td>
<td>Recurrent H&amp;N cancer / second line</td>
<td>Pem (210)</td>
<td>69</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [31]</td>
<td>Metastatic urothelial cancer / second line</td>
<td>Pem (270)</td>
<td>21.1</td>
<td>8.0</td>
<td>2.1</td>
<td>0.73 (0.002)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy (272)</td>
<td>11.4</td>
<td>5.2</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>III [33]</td>
<td>Metastatic triple negative breast cancer / first line</td>
<td>Pem+chemotherapy (566)</td>
<td>20.2</td>
<td>8.1</td>
<td>0.59 (0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy (281)</td>
<td>13.1</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [34]</td>
<td>Stage II or III triple negative breast cancer neoadjuvant</td>
<td>Pem+Pac/Car (401)</td>
<td>64.8 (pCR)</td>
<td></td>
<td></td>
<td>-0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pac/Car (201)</td>
<td>51.2 (pCR)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ORR, overall response rate; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand-1; NR, not reached; H&N, head and neck; Pac, paclitaxel; Car, carboplatin; pCR, pathologic complete remission.

*aPem+chemotherapy resulted in a significant improvement of PFS compared to chemotherapy alone in patients with combined positive score (PD-L1 status) of 10 or more.

*b pCR is pathological stage ypT0/Tis ypN0.

Table 3. Atezolizumab (Ate) clinical trials results

<table>
<thead>
<tr>
<th>Phase</th>
<th>Population</th>
<th>Therapy (No. of patients)</th>
<th>ORR (%)</th>
<th>OS (mo)</th>
<th>PFS (mo)</th>
<th>HR for OS (p-value)</th>
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</thead>
<tbody>
<tr>
<td>II (single arm) [35]</td>
<td>Platinum failed advanced urothelial cancer / second line</td>
<td>Ate 1,200 mg (315)</td>
<td>15</td>
<td>11.7</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td>II (single arm) [36]</td>
<td>Platinum ineligible advanced urothelial cancer / first line</td>
<td>Ate 1,200 mg (119)</td>
<td>23</td>
<td>15.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [37]</td>
<td>Metastatic NSCLC / first line (high expression of PD-L1)*</td>
<td>Ate 1,200 mg (277)</td>
<td>20.2</td>
<td>8.1</td>
<td>0.59 (0.01)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chemotherapy (277)</td>
<td>13.1</td>
<td>5.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II [38]</td>
<td>Metastatic NSCLC / second line</td>
<td>Ate 1,200 mg (425)</td>
<td>13.8</td>
<td>2.8</td>
<td>0.73 (0.0003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Docetaxel (425)</td>
<td>9.5</td>
<td>4.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ORR, overall response rate; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; NSCLC, non-small cell lung cancer; PD-L1, programmed death ligand-1.

*High expression of PD-L1 defined as ≥1% PD-L1 expression on tumor cells and any level of PD-L1 expression on tumor-infiltrating immune cells, <1% PD-L1 expression on tumor cells, and ≥1% PD-L1 expression on tumor-infiltrating immune cells by SP142 assay.

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immune cells around tumors so that they change to an inflamed tumor [47]. To make an inflamed tumor is essential to increasing the response to PD1 or PD-L1 inhibitors.

1. Combination of chemotherapy

Recently, many studies have been conducted on combination treatment with immunotherapy, including immunotherapy, chemotherapy, targeted therapy, and radiotherapy. In particular, good candidates with combination treatment are included in chemotherapy or targeted therapy, because conventional therapy could help achieve rapid tumor regression, and immune checkpoint blockade could then help sustain the tumor response, inducing a long-lasting immune-mediated reaction [46]. The study of combination treatment with ICI and chemotherapy started with metastatic NSCLC. In the first-line treatment of metastatic NSCLC, PFS and OS improvement showed the pembrolizumab group with pemetrexed and carboplatin compared to conventional chemotherapy regardless of PD-L1 status (HR, 0.49; p < 0.001). In 2018, this combination therapy was first approved by the FDA and is now routinely used as a first-line treatment in metastatic NSCLC [48,49]. Accordingly, the combination of nab-paclitaxel and atezolizumab was recently approved for first-line treatment of triple negative metastatic breast cancer, showing improved OS compared to the nab-paclitaxel only group in PD-L1 positive patients (OS, 25 months vs. 15.5 months) [50]. The PFS of the combination of nab-paclitaxel and atezolizumab group showed the benefit in all populations (7.5 months vs. 5.0 months; HR, 0.62; p < 0.001) and failed to show the benefit of OS in all populations. However, this study proved that adding chemotherapy in immunotherapy improved survival benefit in the PD-L1 positive group compared to immunotherapy alone. The great success of combination therapy in NSCLC and breast cancer has led to more studies on combination treatment with immunotherapy. In metastatic small cell lung cancer (SCLC), combination treatment with atezolizumab, etoposide, and carboplatin revealed better survival benefit compared to conventional chemotherapy with etoposide and carboplatin in IMpower 133 study (OS, 12.3 months vs. 10.3 months; HR, 0.70; p = 0.007) [51]. Similarly, durvalumab with etoposide and platinum improved the OS compared to conventional chemotherapy in metastatic SCLC first-line treatment in the CASPIAN study (OS months, 13.3 vs. 10.3 months; HR, 0.73; p = 0.0047) [52]. In metastatic urothelial cancer, positive results were sustained when reporting the OS improvement of atezolizumab with platinum-containing chemotherapy compared to placebo and platinum-containing chemotherapy in palliative first-line treatment of metastatic urothelial cancer of IMvigor 130 study (PFS: 8.2 months vs. 6.3 months; HR, 0.82; p = 0.007; OS: 16 months vs. 13.4 months; HR, 0.83; p = 0.027) [53]. However, the OS results were not significant. In addition, the study results of pembrolizumab and platinum-containing chemotherapy vs. pembrolizumab-only vs. chemotherapy only were reported in palliative first-line treatment of metastatic urothelial cancer. HR (95% confidence interval [CI]) for pembrolizumab and chemotherapy vs. chemotherapy was only 0.78 (0.65–0.93, p = 0.0033) for PFS and 0.86 (0.72–1.02, p = 0.0407) for OS but, unfortunately, these results were not significant for OS and PFS [54]. PD-1 or PDL-1 inhibitors in urothelial cancer after palliative treatment seemed to have functional limitations. Recently, in the case of stomach cancer, good results were reported in the CheckMate 649 study that showed capcitabine, oxaliplatin, and nivolumab improved OS and PFS compared with conventional chemotherapy in first-line metastatic stomach cancer treatment (OS: HR, 0.80; p = 0.0002; PFS: HR, 0.68; p < 0.0001) [55].

2. Combination of targeted therapy

In targeted immunotherapy agents, good results have been reported, especially in kidney cancer. Improvement of OS and PFS with the VEGF inhibitor axitinib and pembrolizumab compared to sunitinib, a first-line metastatic RCC (mRCC) conventional treatment (OS: HR, 0.68; p = 0.0003; PFS: HR, 0.71; p < 0.001) [56,57]. Similarly, lenvatinib plus pembrolizumab compared to sunitinib in first-line mRCC treatment showed a significant improvement in OS (HR, 0.66; p = 0.005) [58]. Another study of nivolumab and cabozantinib vs. sunitinib in palliative first-line mRCC showed good results in palliative first-line mRCC treat-
ment. HR (95% CI) for nivolumab and cabozantinib vs. sunitinib was 0.51 (0.41–0.64, p < 0.001) for OS and 0.60 (0.40–0.89, p = 0.001) [59]. In endometrial cancer, one of the gynecologic malignancies, targeted agents with immunotherapy have been reported to improve survival compared to conventional chemotherapy. Lenvatinib plus pembrolizumab improved OS compared to doxorubicin and paclitaxel treatment in second-line metastatic endometrial cancer in the Keynote 775 study (HR, 0.56; p < 0.001) [60].

The status of deficient mismatch repair (dMMR) in tumors was investigated in this study, and the study showed that the lenvatinib plus pembrolizumab group improved survival regardless of dMMR. The results of the combination with immunotherapy trials are listed in Table 4. Not only VEGF inhibitors but also other targeted agents including poly ADP-ribose polymerase (PARP) inhibitor and CDK4/6 inhibitor with ICIs have benefited from synergistic PD-1/PD-L1 inhibitors in preclinical and early clinical data [61]. In particular, phase II studies of PARP inhibitors with PD-1/PD-L1 inhibitor combination treatment in ovarian and breast cancer have shown positive results, and the results of ongoing randomized control trials will be expected [62-64].

**Conclusion**

Since 2015, immunotherapy, especially ICI, has become a mainstream cancer treatment. The studies of ICIs showed good responses of various tumor types when applied to many types of cancer. Immunotherapy has a durable response and low toxicity, but immunotherapy alone has a response of 15% to 20% in the cancer patient treatment group. Thus, the challenge of improving the response to immunotherapy was sustained, and we found that the microenvironment in immune cells is very important to act appropriately in cancer. A combination of chemotherapy or targeted therapy with immunotherapy was started on cancer treatment to make an inflamed tumor help increase the response to immunotherapy. Recently, studies on combination therapy reported improved OS and PFS, even though they did not respond to immu-

<table>
<thead>
<tr>
<th>Phase</th>
<th>Population</th>
<th>Therapy (No. of patients)</th>
<th>OS (mo)</th>
<th>HR (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>III [48] Metastatic NSCLC / first line (nonsquamous) (Keynote 189)</td>
<td>Pem+pemetrexed/Car (410)</td>
<td>NR</td>
<td>0.49 (0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pemetrexed/Car (206)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>III [49] Metastatic NSCLC / first line (squamous) (Keynote 407)</td>
<td>Pem+Pac/Car (278)</td>
<td>15.9</td>
<td>0.64 (0.001)</td>
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<tr>
<td></td>
<td>Pac/Car (281)</td>
<td>11.3</td>
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<tr>
<td>III [50] Metastatic triple negative breast cancer / first line (Immunotherapy130)</td>
<td>Ate+nab-Pac (185) (PD-L1 positive)</td>
<td>25</td>
<td>0.62</td>
<td></td>
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<tr>
<td></td>
<td>Nab-Pac (184) (PD-L1 positive)</td>
<td>15.5</td>
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</tr>
<tr>
<td>III [51] Metastatic SCLC / first line (IMpower133)</td>
<td>Ate+EP (201)</td>
<td>12.3</td>
<td>0.7 (0.007)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EP (202)</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [52] Metastatic SCLC / first line (CASPlian)</td>
<td>Durvalumab+EP (268)</td>
<td>13.3</td>
<td>0.73 (0.0047)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EP (269)</td>
<td>10.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [53] Metastatic urothelial cancer / first line (IMvigor 130)</td>
<td>Ate+Gem/Platinum (451)</td>
<td>16</td>
<td>0.83 (vs. chemotherapy) (0.0027)</td>
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<tr>
<td></td>
<td>Ate (362)</td>
<td>13.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gem/Platinum (400)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [54] Metastatic urothelial cancer / first line (Keynote 361)</td>
<td>Pem+Gem/Platinum (351)</td>
<td>17</td>
<td>0.86 (vs. chemotherapy) (0.0407)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pem (307)</td>
<td>15.6</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Gem/Platinum (352)</td>
<td>14.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [55] Metastatic gastric cancer (CheckMate 649)</td>
<td>Nivo+Xelox or Folfox (789)</td>
<td>13.8</td>
<td>0.80 (0.0002)</td>
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</tr>
<tr>
<td></td>
<td>Chemotherapy (792)</td>
<td>11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [57] Metastatic renal cell cancer (Keynote 426)</td>
<td>Pem+axitinib (432)</td>
<td>NR</td>
<td>0.68 (0.0003)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunitinib (429)</td>
<td>35.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [58] Metastatic renal cell cancer / first line (CLEAR)</td>
<td>Pem+lenvatinib(355)</td>
<td>NR</td>
<td>0.66 (0.001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lenvatinib+everolimus (357)</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sunitinib (357)</td>
<td>NR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III [60] Metastatic endometrial cancer / second line (Keynote 775)</td>
<td>Pem+lenvatinib (411)</td>
<td>18.3</td>
<td>0.62 (0.0001)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chemotherapy (416)</td>
<td>11.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

OS, overall survival; HR, hazard ratio; SCLC, small cell lung cancer; NSCLC, non-SCLC; Pem, pembrolizumab; Car, carboplatin; Pac, paclitaxel; NR, not reached; Ate, atezolizumab; EP, etoposide/cisplatin; Gem, gemcitabine; Nivo, nivolumab; Xelox, xeloda/oxaliplatin; Folfox, 5-FU/oxaliplatin.
notherapy. Many trials of combination therapy are ongoing and will be expected to continue the benefits of survival (Table 5). Thus, combining chemotherapy or targeted agents with immunotherapy is one of the leading pathways for cancer treatment.

Notes

Conflicts of interest

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

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References


Usefulness of presepsin in predicting the prognosis of patients with sepsis or septic shock: a retrospective cohort study

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Background: The diagnosis and prediction of prognosis are important in patients with sepsis, and presepsin is helpful. In this study, we aimed to examine the usefulness of presepsin in predicting the prognosis of sepsis in Korea.

Methods: Patients diagnosed with sepsis according to the sepsis-3 criteria were recruited into the study and classified into surviving and non-surviving groups based on in-hospital mortality. A total of 153 patients (32 and 121 patients with sepsis and septic shock, respectively) were included from July 2019 to August 2020.

Results: Among the 153 patients with sepsis, 91 and 62 were in the survivor and non-survivor groups, respectively. Presepsin ($p=0.004$) and lactate ($p=0.003$) levels and the sequential organ failure assessment (SOFA) score ($p<0.001$) were higher in the non-survivor group. Receiver operating characteristic curve analysis revealed poor performances of presepsin and lactate in predicting the prognosis of sepsis (presepsin: area under the curve [AUC] =0.656, $p=0.001$; lactate: AUC =0.646, $p=0.003$). The SOFA score showed the best performance, with the highest AUC value (AUC =0.751, $p<0.001$). The prognostic cutoff point for presepsin was 1,176 pg/mL. Presepsin levels higher than 1,176 pg/mL (odds ratio [OR], 3.352; $p<0.001$), higher lactate levels (OR, 1.203; $p=0.003$), and higher SOFA score (OR, 1.249; $p<0.001$) were risk factors for in-hospital mortality.

Conclusion: Presepsin levels were higher in non-survivors than in survivors. Thus, presepsin may be a valuable biomarker in predicting the prognosis of sepsis.

Keywords: Biomarkers; Prognosis; Sepsis; Septic shock

Introduction

Sepsis is a condition that is accompanied by systemic inflammatory reactions caused by infection and major organ failure [1]. If not detected and treated quickly, the mortality rate reaches 40% to 70% [2,3]. The treatment of sepsis requires the key elements of intensive care unit (ICU) treatment, such as hemodynamic monitoring and support, ventilator therapy, and renal replacement therapy [4]. It is well known that the early diagnosis and proper treatment of sepsis can improve prognosis and increase the survival of people with sepsis [5].

In 2001, the sepsis mortality rate in the United States was 28.6% and has recently declined to 20% [6]. Moreover, the sepsis mortality rate in Australia and New Zealand has been reported to be 18.4% [7]. Unfortunately, the sepsis mortality rate in Korea is far higher than that in developed countries. Currently, the mortality
rate of sepsis in Korea, as reported by the Korean Society of Critical Care Medicine in 2013, is 37.8%, and the number of deaths from sepsis in the same year was 15,076, which constituted a large percentage of total deaths [2]. Moreover, in Korea, as the number of elderly and immunocompromised patients increases rapidly, the social burden from sepsis is expected to increase significantly in the near future.

Among various molecules, presepsin appears to be a promising biomarker, as it has been reported to be involved in the early stages of the septic process [8]. When monocytes are activated by an infectious agent, the soluble CD14 subtype, presepsin, is released into the plasma [9]. Subsequently, presepsin levels continue to increase in the early stages of sepsis. In Korea, presepsin levels were significantly higher in the infected group than in the uninfected group [10]. In this study, we examined whether presepsin levels are an effective marker for predicting prognosis in patients with sepsis or septic shock.

**Materials and methods**

1. Study population and study design

All study data were retrieved from electronic medical records (C&U care, Daejeon, Korea). Patients who were diagnosed with sepsis or septic shock by a physician and those whose presepsin levels were checked were included in the study. Sepsis was defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Organ dysfunction was identified as an acute change in the total sequential organ failure assessment (SOFA) score of ≥ 2 points consequent to the infection. Septic shock was defined based on the sepsis-3 criteria as the presence of the following conditions: (1) sepsis, (2) need for vasopressor/inotropes to maintain mean blood pressure of ≥ 65 mmHg, and (3) lactate of > 2 mmol/L, even with sufficient fluids [1]. For this study, we included all adult patients who were admitted to the ICU directly from the emergency department or ward. The inclusion criteria were: more than 18 years of age, diagnosis of sepsis or septic shock, and presepsin levels measured. Exclusion criteria were: not admitted to the ICU, presepsin levels not measured, and incomplete data. A total of 153 patients were included in the period between July 2019 and August 2020 (Fig. 1).

Age, sex, laboratory data (white blood cell [WBC] and platelet counts, total bilirubin, creatinine, procalcitonin, and lactate levels), and blood samples were collected from patients with sepsis or septic shock. The presepsin levels in the blood samples were measured using an automated analyzer (LSI Medience Corp., Tokyo, Japan) with a detection range of 20 to 20,000 pg/mL. Clinical data related to patient prognosis were collected during the hospital stay. The patients were observed from the date of admission to the date of discharge or death. Subsequently, a retrospective analysis was performed.

2. Statistical analysis

All values are expressed as mean ± standard deviations for continuous variables and as percentages for categorical variables. Student t-test or Mann-Whitney U-test was used for continuous data, and Pearson chi-squared test or Fisher exact test was used for categorical data. Receiver operating characteristic (ROC) curve analysis was performed to evaluate prediction accuracy. The optimal cutoff value was chosen as the highest product of sensitivity and specificity. Univariate logistic regression analysis was performed to identify predictors of in-hospital mortality. All p-values were two-tailed, with statistical significance set at p < 0.05. All the statistical analyses were performed using IBM SPSS version 22.0 (IBM Corp., Armonk, NY, USA).

**Results**

1. Baseline characteristics of patients

The baseline characteristics of the 153 studied patients are shown in Table 1. The non-survivor group had older patients (72.2 ± 12.5 years vs. 67.0 ± 14.6 years, p = 0.025) and higher 30-day mortality (72.6% vs. 0%, p < 0.001); additionally, the number of patients receiving ventilatory assistance during the ICU stay was higher in the non-survivor group than in the survivor group (87.1% vs. 47.3%, p < 0.001) (Table 1).

The presepsin (3,112 ± 3,841 pg/mL vs. 1,511 ± 2,092 pg/mL, p = 0.004) and lactate (4.3 ± 3.3 mmol/L vs. 2.8 ± 2.4 mmol/L, p = 0.003) levels and the SOFA score (12 ± 4 vs. 8 ± 4, p < 0.001) were higher in the non-survivor group than in the survivor group.
Table 1. Patients’ baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total</th>
<th>Survivor group</th>
<th>Non-survivor group</th>
<th>p-valuea</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>153</td>
<td>91</td>
<td>62</td>
<td>0.025</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>69.1±14.0</td>
<td>67.0±14.6</td>
<td>72.2±12.5</td>
<td>0.025</td>
</tr>
<tr>
<td>Male sex</td>
<td>94 (61.4)</td>
<td>59 (64.8)</td>
<td>35 (56.5)</td>
<td>0.296</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.7±12.7</td>
<td>24.3±16.3</td>
<td>22.9±4.2</td>
<td>0.515</td>
</tr>
<tr>
<td>Laboratory value</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC (µL)</td>
<td>13,285±9,164</td>
<td>13,212±8,642</td>
<td>13,393±9,953</td>
<td>0.905</td>
</tr>
<tr>
<td>Platelets (×10³/µL)</td>
<td>154±108</td>
<td>168±109</td>
<td>133±103</td>
<td>0.052</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>2.0±3.0</td>
<td>1.6±2.3</td>
<td>2.7±3.8</td>
<td>0.044</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.6±1.4</td>
<td>1.6±1.6</td>
<td>1.8±1.3</td>
<td>0.415</td>
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<tr>
<td>CRP (mg/dL)</td>
<td>15.5±10.2</td>
<td>16.1±10.1</td>
<td>14.7±10.3</td>
<td>0.414</td>
</tr>
<tr>
<td>Procalcitonin (ng/mL)</td>
<td>18.9±42.5</td>
<td>16.4±40.3</td>
<td>22.7±45.7</td>
<td>0.378</td>
</tr>
<tr>
<td>Presepsin (pg/mL)</td>
<td>2,159±3,022</td>
<td>1,511±2,092</td>
<td>3,112±3,841</td>
<td>0.004</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>3.4±2.9</td>
<td>2.8±2.4</td>
<td>4.3±3.3</td>
<td>0.003</td>
</tr>
<tr>
<td>Severity of sepsis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>APACHE II score</td>
<td>19±10</td>
<td>18±8</td>
<td>21±11</td>
<td>0.051</td>
</tr>
<tr>
<td>SOFA score</td>
<td>10±4</td>
<td>8±4</td>
<td>12±4</td>
<td>&lt;0.001</td>
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<tr>
<td>Sepsis</td>
<td>32 (20.9)</td>
<td>21 (23.1)</td>
<td>11 (17.7)</td>
<td>0.342</td>
</tr>
<tr>
<td>Septic shock</td>
<td>121 (79.1)</td>
<td>70 (76.9)</td>
<td>51 (82.3)</td>
<td>0.426</td>
</tr>
<tr>
<td>30-Day mortality</td>
<td>45 (29.4)</td>
<td>0 (0)</td>
<td>45 (72.6)</td>
<td>&lt;0.001</td>
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<td>Treatment during ICU stay</td>
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<td></td>
<td></td>
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<tr>
<td>Mechanical ventilation</td>
<td>97 (63.4)</td>
<td>43 (47.3)</td>
<td>54 (87.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vasopressors</td>
<td>109 (71.2)</td>
<td>61 (67.0)</td>
<td>48 (77.4)</td>
<td>0.163</td>
</tr>
</tbody>
</table>

Values are presented as number only, mean±standard deviation, or number (%).

WBC, white blood cell; CRP, C-reactive protein; APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, sequential organ failure assessment; ICU, intensive care unit.

a) Analyzed between survivor and non-survivor groups.

(Fig. 1). However, there was no significant difference in the WBC counts (13,212±8,642/µL vs. 13,393±9,953/µL, p = 0.905), C-reactive protein (CRP) (16.1±10.1 mg/dL vs. 14.7±10.3 mg/dL, p = 0.414) levels, procalcitonin levels (16.4±40.3 ng/mL vs. 22.7±45.7 ng/mL, p = 0.378), and Acute Physiology and Chronic Health Evaluation II (APACHE II) score (18±8 vs. 21±11, p = 0.051) between the two groups (Fig. 2).

2. Comparison of the infection sources and pathogens between the two groups

The frequency of urinary tract infection was lower in the non-survivor group (6.5% vs. 18.7%, p = 0.031) than in the survivor group. There was no significant difference in the other sources of infection (Table 2).

The frequency of fungal infections was higher (9.7% vs. 0%, p = 0.002) in the non-survivor group than in the survivor group, while those of bacterial and viral infections were not significantly different between the two groups (Table 2).

3. Predictors of patients’ in-hospital mortality

A binary logistic regression analysis showed that a presepsin levels higher than 1,176 pg/mL (odds ratio [OR], 3.352; 95% confidence interval [CI], 1.707–6.585; p < 0.001), higher lactate levels (OR, 1.203; 95% CI, 1.063–1.361; p = 0.003), and higher SOFA score (OR, 1.249; 95% CI, 1.136–1.373; p < 0.001) were risk factors for in-hospital mortality due to sepsis (Table 3). However, there was no association between in-hospital mortality and WBC, CRP, and procalcitonin levels. When presepsin was used as a continuous variable, there was no meaningful value with OR of 1.000 (95% CI, 1.000–1.000; p = 0.047). However, when the cutoff value was applied, a meaningful value was confirmed for presepsin.

4. Prognostic value of the sepsis biomarker and the SOFA score

The performances of the levels of WBC, CRP, procalcitonin, presepsin, lactate, and SOFA score in predicting in-hospital mortality from sepsis were evaluated using ROC curves (Fig. 3). The levels of presepsin and lactate demonstrated poor performance in predicting the prognosis of sepsis (presepsin: area under the curve...
Fig. 2. Vertical box-and-whiskers plots summarizing laboratory data on all bombarded construct combinations. The whiskers indicate the 5th–95th percentile: any data points outside of this are shown as dots. (A) White blood cells (WBC) counts, (B) C-reactive protein (CRP), (C) procalcitonin level, (D) presepsin levels, (E) lactate levels, and (F) the sequential organ failure assessment (SOFA) score in the survivor and non-survivor groups.

Table 2. Infection sources and pathogens of patients

| Variable          | Total (n = 153) | Survivor group (n = 91) | Non-survivor group (n = 62) | p-value
<table>
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<tbody>
<tr>
<td>Sources of infection</td>
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</tr>
<tr>
<td>Respiratory tract</td>
<td>92 (60.1)</td>
<td>54 (59.3)</td>
<td>38 (61.3)</td>
<td>0.809</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>21 (13.7)</td>
<td>17 (18.7)</td>
<td>4 (6.5)</td>
<td>0.031</td>
</tr>
<tr>
<td>Intra-abdomen</td>
<td>19 (12.4)</td>
<td>9 (9.9)</td>
<td>10 (16.1)</td>
<td>0.251</td>
</tr>
<tr>
<td>Others^b</td>
<td>23 (15.0)</td>
<td>12 (13.2)</td>
<td>11 (17.7)</td>
<td>0.439</td>
</tr>
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<td>Pathogens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram (-) rods</td>
<td>30 (19.6)</td>
<td>21 (23.1)</td>
<td>9 (14.5)</td>
<td>0.190</td>
</tr>
<tr>
<td>Gram (-) bacilli</td>
<td>37 (24.2)</td>
<td>15 (16.5)</td>
<td>9 (14.5)</td>
<td>0.743</td>
</tr>
<tr>
<td>Gram (+) cocci</td>
<td>37 (24.2)</td>
<td>24 (26.4)</td>
<td>13 (21.0)</td>
<td>0.443</td>
</tr>
<tr>
<td>Gram (+) bacilli</td>
<td>5 (3.3)</td>
<td>1 (1.1)</td>
<td>4 (6.5)</td>
<td>0.068</td>
</tr>
<tr>
<td>Fungus</td>
<td>6 (3.9)</td>
<td>0 (0)</td>
<td>6 (9.7)</td>
<td>0.002</td>
</tr>
<tr>
<td>Virus</td>
<td>5 (3.3)</td>
<td>5 (5.5)</td>
<td>0 (0)</td>
<td>0.061</td>
</tr>
<tr>
<td>Negative cultures</td>
<td>47 (30.7)</td>
<td>27 (29.7)</td>
<td>20 (32.3)</td>
<td>0.733</td>
</tr>
</tbody>
</table>

Values are presented as number (%).
If there are multiple sources of infection and/or pathogens in one patient, all of them are shown in the table. Analyzed between survivor and non-survivor groups. Other site: skin, bone, and surgical site infection.

\[ \text{AUC} = 0.656, \ p = 0.001; \ \text{lactate: AUC} = 0.646, \ p = 0.003 \]. The SOFA score had the best performance, with the highest AUC value (AUC = 0.751, \( p < 0.001 \)). The prognostic cutoff value for presepsin was 1,176 pg/mL, at which the sensitivity and specificity were 66.7% and 61.1%, respectively. In addition, the prognostic cutoff value of the SOFA score was 9.50 (sensitivity, 72.6%; specificity, 67.0%), and the prognostic cutoff value of lactate was 1.55 (sensitivity, 91.8%; specificity, 34.8%). The Pearson correlation coefficient of the SOFA score and presepsin was 0.421 (\( p < 0.001 \)), which had a moderate quantitative linear relationship.
Discussion

The patients were divided into two groups, and a retrospective analysis was performed according to in-hospital mortality. We evaluated the performance of presepsin as a biomarker for predicting in-hospital mortality in patients with sepsis. Presepsin levels were higher in the non-survivor group than in the survivor group. The levels of presepsin displayed poor performance in predicting the prognosis of sepsis (presepsin: AUC = 0.656, \( p = 0.001 \)), and the prognostic accuracy of presepsin was 1,176 pg/mL, with a sensitivity and specificity of 66.7% and 61.1%, respectively. Presepsin levels higher than 1,176 pg/mL were a significant predictor of in-hospital mortality.

Presepsin is a 13-kDa glycoprotein-truncated N-terminal fragment of CD14 that activates the pro-inflammatory signaling stage after it is released into the circulation following contact with an infectious pathogen [11,12]. Presepsin was first detected by biochemical methods and has been considered a new biomarker for infection in Japan [13]. A meta-analysis in 2015 confirmed its diagnostic accuracy [11,14], and the prognostic accuracy of presepsin in sepsis has been reported in several clinical studies [8,10,15-19]. In Korea, presepsin levels were significantly higher in the infected group than in the uninfected group. Moreover, the diagnostic accuracy of presepsin is higher than that of other conventional biomarkers [10].

Sepsis is a common disease in hospitalized patients with high morbidity and mortality rates. Therefore, several factors have been studied to enhance the rapid diagnosis of sepsis and predict prognosis. Procalcitonin [20], lactate [21], and interleukin-6 levels are prognostic laboratory markers of sepsis [22,23]. Presepsin has also been studied as a prognostic factor [19].

In this study, the levels of presepsin (3,112 ± 3,841 pg/mL vs. 1,511 ± 2,092 pg/mL, \( p = 0.004 \)) were found to be higher in the non-survivor group than in the survivor group. However, presepsin demonstrated poor performance in predicting the prognosis of sepsis (presepsin: AUC = 0.656, \( p = 0.001 \)), as confirmed by the ROC curve analysis in this study. This study showed that the mean age of patients in the non-survivor group was higher than that of the survivor group. Giavarina et al. [24] reported that presepsin concentrations were higher in patients aged ≥ 70 years compared...
to younger patients (87% vs. 47%, p < 0.001). This result suggests that presepsin levels might be altered and have relatively poor predictive potential in elderly patients with sepsis in the ICU. However, several studies have shown that presepsin levels are higher in non-survivors among patients with sepsis, and some studies have shown presepsin to be a statistically significant prognostic factor. Moreover, Wen et al. [18] showed that presepsin levels in the non-survivor group were significantly higher (1,692 pg/mL [342–20,000 pg/mL] vs. 1,125 pg/mL [484–2,268 pg/mL], p = 0.000), and that presepsin levels were an independent risk factor for in-hospital mortality in patients with sepsis (OR, 1.221; p = 0.026). ROC curve analysis showed that presepsin levels are good predictors of in-hospital mortality in patients with sepsis (AUC = 0.703, p = 0.000) [18]. Furthermore, Jereb et al. [25] showed that presepsin levels were higher in patients with septic shock than in patients with sepsis (1,914 pg/mL [342–20,000 pg/mL] vs. 771 pg/mL [286–5,565 pg/mL], p < 0.01). Mean presepsin concentrations were higher in the non-survivors (1,941 pg/mL [342–20,000 pg/mL] vs. 1,208 pg/mL [286–12,096 pg/mL], p = 0.009). The trend of changes in presepsin concentrations in the deceased patients was significantly different from that in the surviving patients (p = 0.018) [25]. Masson et al. [26] showed that the presepsin levels were higher in decedents (2,269 pg/mL [1,171–4,300 pg/mL]) than in survivors (1,184 pg/mL [875–2,113 pg/mL], p = 0.002). The evolution of presepsin levels over time was significantly different between survivors and decedents (p for time-survival interaction = 0.03). Furthermore, presepsin levels were associated with ICU stay and 28-day mortality (hazard ratio, 1.55; 95% CI, 1.12–2.13; p = 0.008) in Cox models adjusted for clinical characteristics [26]. The cutoff values of presepsin levels that predict mortality in patients with sepsis have been identified to lie between 556 and 2,455 ng/L [17,19,27–29]. However, in some studies, presepsin levels did not show any significance in predicting mortality [10,16,28]. In other studies, the prognostic value of presepsin was different, and the cutoff value was not clear [7,17,25,30]. It is thought that the non-severe patient groups were not comparable; the differences in the underlying disease and the degree of infection influenced the outcome. In this study, the number of patients was small, and patients with underlying hematologic malignancy were included; therefore, it is thought that this may have influenced the study results. Considering these points, it is believed that further studies involving a larger number of patients with a similar underlying disease would be helpful in identifying the prognostic factors.

There are some limitations to our study. First, the sample size was relatively small. The number of patients included in each group was relatively small, and no bacterial, fungal, or viral etiologies were identified. Therefore, a large-scale multicenter study is needed. Second, this was a single-center study that could have led to selection biases. Third, patients’ presepsin levels were measured only during hospitalization and were not monitored daily.

In conclusion, the diagnosis of sepsis and prediction of prognosis are important. Although scoring systems such as the SOFA score provide assistance, the scores need to be calculated by the clinicians; hence, the score cannot be checked immediately. Thus, the importance of laboratory markers that can quickly identify sepsis is increasing. Presepsin levels were higher in the deceased group, and mortality was predictable at a cutoff level of 1,716 mg/mL. However, the ROC curve did not show any significant values. Additional studies, such as multicenter studies, would be helpful.

Notes

Ethical statements

This study was approved by the Institutional Review Board (IRB) of Chungnam University (IRB No: CNUH 2020-06-074), and the requirement for informed consent was waived because of the retrospective nature of the study.

Conflicts of interest

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

Author contributions

Conceptualization, Data curation: JSK, SIL; Formal analysis: JSK, YJK, SIL; Methodology: JSK, JEL, SIL; Visualization: JSK, DHK, SIL; Writing-original draft: JSK, SIL; Writing-review & editing: all authors.

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29. Carpio R, Zapata J, Spanuth E, Hess G. Utility of presepsin (sCD14-ST) as a diagnostic and prognostic marker of sepsis in

Sulfatase 1 and sulfatase 2 as novel regulators of macrophage antigen presentation and phagocytosis

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Background: Sulfation of heparan sulfate proteoglycans (HSPGs) is critical for the binding and signaling of ligands that mediate inflammation. Extracellular 6-O-endosulfatases regulate post-translational sulfation levels and patterns of HSPGs. In this study, extracellular 6-O-endosulfatases, sulfatase (Sulf)-1 and Sulf-2, were evaluated for their expression and function in inflammatory cells and tissues.

Methods: Harvested human peripheral blood mononuclear cells were treated with phytohemagglutinin and lipopolysaccharide, and murine peritoneal macrophages were stimulated with interleukin (IL)-1β for the evaluation of Sulf-1 and Sulf-2 expression. Sulf expression in inflammatory cells was examined in the human rheumatoid arthritis (RA) synovium by immunofluorescence staining. The antigen presentation and phagocytic activities of macrophages were compared according to the expression state of Sulfs. Sulfs-knockdown macrophages and Sulfs-overexpressing macrophages were generated using small interfering RNAs and pcDNA3.1 plasmids for Sulf-1 and Sulf-2, respectively.

Results: Lymphocytes and monocytes showed weak Sulf expression, which remained unaffected by IL-1β. However, peritoneal macrophages showed increased expression of Sulfs upon stimulation with IL-1β. In human RA synovium, two-colored double immunofluorescent staining of Sulfs and CD68 revealed active upregulation of Sulfs in macrophages of inflamed tissues, but not in lymphocytes of lymphoid follicles. Macrophages are professional antigen-presenting cells. The antigen presentation and phagocytic activities of macrophages were dependent on the level of Sulf expression, suppressed in Sulfs-knockdown macrophages, and enhanced in Sulfs-overexpressing macrophages.

Conclusion: The results demonstrate that upregulation of Sulfs in macrophages occurs in response to inflammation, and Sulfs actively regulate the antigen presentation and phagocytic activities of macrophages as novel immune regulators.

Keywords: Antigen presentation; Macrophages; Sulfatase 1; Sulfatase 2

Introduction

Heparan sulfate proteoglycans (HSPGs) are ubiquitous molecules on the cell surface and matrix in all animal species. They act as receptors, co-receptors, reservoirs, or inhibitors of various ligands, and have many biological implications by binding to growth factors, cytokines, chemokines, adhesion molecules, extracellular matrix components, degradative enzymes, protease inhibitors, and
proteins involved in lipid metabolism [1].

Accumulating evidence suggests that various proinflammatory cytokines can upregulate the surface expression of specific HSPGs and heparin/heparan sulfates (HS), which participate in inflammatory responses and modulate the interactions with leukocytes [2]. Upon binding to cell surfaces, matrix proteins, and soluble ligands, HSPGs can regulate inflammatory cell maturation and activation, leukocyte rolling, adhesion, extravasation, and chemotaxis [3].

The biological functions of HSPGs can be attributed to the specialized structures within the HS moieties [4]. Posttranslational modifications of HSPGs, such as sulfation, give rise to the molecular diversity and heterogeneity of HSPGs. Cytokines and chemokines bind selectively to the substructures of HSPGs, which determine the specificity of leukocyte recruitment [5-8]. Sulfation of HSPGs is critical for binding and signaling of the ligands that mediate inflammation and is essential for leukocyte rolling and adhesion to regulate inflammation [9]. Antigen-specific B cell differentiation can also be affected by the size of glycosaminoglycans (GAGs) and sulfation of HSPGs [10-12].

The pattern of HSPG sulfation is enzymatically modified by various sulfotransferases and sulfatases (Surfs). Surfs, which are enzymes of the esterase class, catalyze the hydrolysis of sulfate esters and participate in the degradation and modulation of sulfated GAGs in the lysosome [13]. Extracellular 6-O-endosulfatases, recently identified as Sulf-1 and Sulf-2 aryl-sulfatases, regulate posttranslational sulfation levels and patterns of HSPGs in extracellular compartments. They selectively remove 6-O-sulfate groups from HSPGs via intramolecular hydrolysis and rearrangement [14-17].

A large amount of data is available regarding HSPGs related to inflammation; however, the effects of modification by sulfation remain unclear. This study was conducted to investigate the cellular expression of Surfs and their role in inflammation.

Materials and methods

1. Reagents

Easy-blue total RNA extraction kit for total RNA isolation was purchased from iNtRON Biotechnology (Seoul, Korea). Dulbecco’s phosphate-buffered saline, penicillin-streptomycin, and fetal bovine serum (FBS) were purchased from Gibco/BRL (Life Technologies, Gaithersburg, MD, USA). Ovalbumin (OVA), phytohemagglutinin (PHA), and lipopolysaccharides (LPS) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Interleukin (IL)-1β and monocyte chemotactic protein-1 (MCP-1) were obtained from R&D Systems (Minneapolis, MN, USA). The Qiagen Mini kit was purchased from Qiagen Sciences (Germantown, MD, USA). LightCycler FastStart DNA SYBR Green I Mix was obtained from Roche (Mannheim, Germany). Rabbit anti-human Sulf-1 polyclonal antibody and rabbit anti-human Sulf-2 were purchased from Santa Cruz Biotechnology (CA, USA). Antibodies against CD68 were purchased from Abcam Inc. (Cambridge, MA, USA); goat anti-mouse immunoglobulin G (IgG) Alexa Fluor 488, goat anti-rabbit IgG Alexa Fluor 546, rabbit anti-goat IgG Alexa Fluor 546, normal goat serum, and normal rabbit serum from Invitrogen Corp. (Carlsbad, CA, USA). Primer sequences for Sulf-1, Sulf-2, and β-actin were synthesized by Bionics (Daegu, Korea). Sulf-1 and Sulf-2 small interfering RNA (siRNA) sequences were purchased from Santa Cruz Biotechnology. Negative control siRNA was purchased from Invitrogen (Carlsbad). pcDNA3.1(−) vector for transfection was obtained from Invitrogen Life Technologies (Carlsbad, CA, USA). pcDNA3.1/myc-His(−) Sulf-1 and pcDNA3.1/myc-His(−) Sulf-2 from Addgene Inc. (Cambridge, MA, USA).

2. Procurement of rheumatoid arthritis synovial tissues and immunofluorescence staining

With informed consent, synovial tissues were obtained from patients with rheumatoid arthritis (RA) who underwent total knee replacement surgery at Yeungnam University Medical Center. A CD68 primary antibody specific to macrophages and anti-Sulf-1, Sulf-2 primary antibodies were applied in parallel for immunofluorescence after pretreatment for paraffin sections and blocking. After overnight incubation with the primary antibodies at 4°C, the sections were treated with the respective secondary antibodies labeled with fluorescence for 30 minutes avoiding light at room temperature. Cell nuclei were counterstained with Hoechst 33258 diluted at 1:5,000 in distilled water for 2 minutes protected from light at room temperature. Expression of Sulf-1, Sulf-2, and CD68 was observed using a fluorescence microscope, and the images were merged for double staining using Photoshop 7.0 (Adobe Systems Inc., San Jose, CA, USA).

3. Human peripheral blood mononuclear cells preparation

Peripheral blood mononuclear cells (PBMCs) were isolated by endotoxin-free Ficoll-Paque PLUS centrifugation (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) from buffy coats obtained from healthy adult blood donors at Yeungnam University Medical Center. PBMCs were cultured at a cell concentration of 10^6 cells/mL in RPMI 1640 medium (Lonza, Basel, Switzerland) supplemented with 10% FBS and 1% penicillin-streptomycin. Prior to stimulation, plates were incubated for 1.5 hours (37°C, 5% CO₂, 100% humidity).
4. Mouse peritoneal macrophage preparation
Specific pathogen-free, 7-week-old inbred BALB/c mice (six mice, three experiments/mouse) were purchased from Central Lab Animal Inc. (Seoul, Korea). The utmost precautions were taken to ensure that the mice remained free from infection by environmental pathogens. The mice were cared for in accordance with the principles of the Guide to the Care and Use of Experimental Animals of Yeungnam Medical Center. Peritoneal macrophages were obtained by lavage using Hanks’ balanced salt solution (HBSS) 4 days after injection with 2 mL of 4% thioglycollate medium (BBL-Becton Dickinson, Cockeysville, MD, USA). Macrophages in complete medium (RPMI 1640 supplemented with 1% penicillin-streptomycin and 10% FBS) were plated in six well tissue culture plates, incubated for 2 hours at 37°C in an atmosphere of 5% CO₂, and then washed three times with HBSS to remove any non-adherent cells. Macrophages were cultured overnight in a complete medium at 37°C in 5% CO₂. The medium was then replaced with serum-free RPMI 1640, and the cells were cultured in the presence or absence of stimuli for the indicated times.

5. Sulf-1, Sulf-2 knockdown
Macrophages were transfected with Sulf-1 and Sulf-2 siRNA oligomers (50 nmol/L) using Lipofectamine 2000 (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer’s instructions. After 24 hours of incubation, the macrophages were placed in a growth medium for 24 hours before the experiments.

6. Sulf-1, Sulf-2 overexpression
pcDNA3.1, pcDNA3.1/Sulf-1, and pcDNA3.1/Sulf-2 plasmids were amplified after transformation into MAX Efficiency DH5α-Competent cells on LB medium with 100 µg/mL ampicillin. The amplified plasmids were purified from cell lysates using the Qiagen Mini kit, according to the manufacturer’s instructions. One microgram of total RNA per sample was reverse-transcribed using a Maxime RT premix kit (iNtRON Biotechnology, Daejeon, Korea) according to the manufacturer’s instructions. cDNA synthesis was performed at 45°C for 60 minutes, followed by real-time (RT) inactivation at 95°C for 5 minutes. Sulf-1 and Sulf-2 were amplified by RT polymerase chain reaction (PCR) using a LightCycler (Roche). The total PCR volume was 20 µL, and each PCR reaction consisted of LightCycler FastStart DNA SYBR Green I mix (Roche), primer, and 2 µL of cDNA. Prior to PCR amplification, the mixture was incubated at 9°C for 10 minutes. The amplification step consisted of 45 cycles of denaturation (10 seconds at 95°C), annealing (5 seconds at the primer-appropriate temperature), and extension (10 seconds at 72°C) with fluorescence detection at 72°C after each cycle. After the final cycle, melting point analyses of all samples were performed over a temperature range of 65°C–95°C with continuous fluorescence detection. β-Actin was used as a reference gene for the normalization of sample expression levels. The primers used for PCR were as follows: Sulf-1 (195 bp) sense, 5′-tcgaacctgccctgagcaga-3′, antisense, 5′-tcgatcgaacctgccctgag-3′; Sulf-2 (195 bp) sense, 5′-tcaagtgaacagccccggactca-3′, antisense, 5′-agcaaccttcttcggtgcttc-3′; and β-actin (148 bp) sense, 5′-agagaaaggctggcagtgt-3′, antisense, 5′-caagacttgagctggccgt-3′. The mRNA levels of Sulf-1 and Sulf-2 were determined by comparing experimental levels to standard curves and were expressed as relative fold expression levels.

8. Phagocytosis assay
Mouse peritoneal macrophages were transfected with siRNA oligomers or control plasmid DNA. After 24 hours of incubation, a phagocytosis assay using a Cytoselect 96-well phagocytosis assay kit was performed according to the manufacturer’s instructions (Cell Biolabs, San Diego, CA, USA). Images of phagocytic macrophages were taken with a Leica DFC 495 camera (Leica Microsystems, Cambridge, UK) mounted on a Nikon microscope (Eclipse TE300; Nikon, Tokyo, Japan) at 200× magnification.

9. Real-time polymerase chain reaction
Total RNA was extracted using the easy-BLUE Total RNA Extraction Kit (iNtRON Biotechnology, Seoul, Korea) according to the manufacturer’s instructions. One microgram of total RNA per sample was reverse-transcribed using a Maxime RT premix kit (iNtRON Biotechnology, Daejeon, Korea) according to the manufacturer’s instructions. cDNA synthesis was performed at 45°C for 60 minutes, followed by real-time (RT) inactivation at 95°C for 5 minutes. Sulf-1 and Sulf-2 were amplified by RT polymerase chain reaction (PCR) using a LightCycler (Roche). The total PCR volume was 20 µL, and each PCR reaction consisted of LightCycler FastStart DNA SYBR Green I mix (Roche), primer, and 2 µL of cDNA. Prior to PCR amplification, the mixture was incubated at 9°C for 10 minutes. The amplification step consisted of 45 cycles of denaturation (10 seconds at 95°C), annealing (5 seconds at the primer-appropriate temperature), and extension (10 seconds at 72°C) with fluorescence detection at 72°C after each cycle. After the final cycle, melting point analyses of all samples were performed over a temperature range of 65°C–95°C with continuous fluorescence detection. β-Actin was used as a reference gene for the normalization of sample expression levels. The primers used for PCR were as follows: Sulf-1 (195 bp) sense, 5′-tcgaacctgccctgagcaga-3′, antisense, 5′-tcgatcgaacctgccctgag-3′; Sulf-2 (195 bp) sense, 5′-tcaagtgaacagccccggactca-3′, antisense, 5′-agcaaccttcttcggtgcttc-3′; and β-actin (148 bp) sense, 5′-agagaaaggctggcagtgt-3′, antisense, 5′-caagacttgagctggccgt-3′. The mRNA levels of Sulf-1 and Sulf-2 were determined by comparing experimental levels to standard curves and were expressed as relative fold expression levels.

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10. Western blotting

Total lysates were prepared in PRO-PREP buffer (iNtRON Biotechnology, Seoul, Korea). Protein concentrations were determined by Bradford assay (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard. Twenty micrograms of protein samples were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and then transferred onto nitrocellulose membranes. Membranes were soaked in 5% non-fat dried milk in TBST (10 mmol/L Tris/HCl pH 7.5, 150 mmol NaCl, and 0.05% Tween-20) for 1 hour, followed by incubation for 16 to 18 hours with primary antibodies against Sulf-1, Sulf-2, and β-actin at 4°C. Membranes were then washed three times with TBST for 10 minutes, followed by incubation with horseradish peroxidase-conjugated secondary antibody for 1 hour at room temperature. Finally, membranes were rinsed three times with TBST for 10 minutes, and antigen-antibody complexes were detected using an enhanced chemiluminescence detection system (LAS-3000; Fujifilm, Tokyo, Japan).

11. Statistical analyses

Statistical analysis was performed using SPSS version 12.0 (SPSS Inc., Chicago, IL, USA). Independent sample t-test was used for parametric analysis, and Mann-Whitney test was used for nonparametric analysis with a confidence interval of 95% and p-value of < 0.05.

Results

HSPGs are involved in signal transduction and may play a role in inflammation. The effects of HSPGs can be modified by the sulfation pattern and level, which is posttranslationally regulated by Sulf-1 and Sulf-2.

In this study, we evaluated Sulf-1 and Sulf-2 expression in inflammatory cells. After harvesting PBMCs from healthy volunteers, the cells were treated with PHA and LPS to activate lymphocytes and monocytes, respectively. Regarding tissue macrophages, murine peritoneal macrophages were stimulated with IL-1β, the key proinflammatory cytokine, and MCP-1, which regulates the migration and infiltration of monocytes and macrophages. Normal non-treated (NT) PBMCs showed weak expression of Sulfs, showing faint bands on western blot (WB), whereas IL-1β-stimulated SW1353 showed increased expression of Sulf-1 and Sulf-2 as a positive control. Compared with the NT control, human lymphocytes and monocytes showed no change in Sulf expression upon in vitro activation. However, murine peritoneal macrophages showed increased expression of Sulf-1 and Sulf-2 following stimulation with IL-1β (Fig. 1A).

The effects of inflammation on the expression of Sulf-1 and Sulf-2 in inflammatory cells were examined in the human RA synovium. RA synovial tissues showed chronic inflammatory reactions of active proliferative villi, hyperplastic fibroblast-like synoviocytes (FLSs) invading sublining tissues, densely infiltrated inflammatory cells forming lymphoid follicles, increased vasculature, thickened interstitium, and fibrosis. Synovial macrophages were recognizable as CD68 positive cells infiltrated into the sublining layers. Lymphocytes and mononuclear cells were identified as CD68 negative cells of small nuclei clustered in lymphoid follicles. Two-colored double immunofluorescence staining of Sulfs and CD68 was performed to verify the inflammatory cells expressing Sulf-1 and Sulf-2 in RA synovial tissue.

RA synovial tissues were incubated with anti-CD68 antibody, followed by incubation with Alexa Fluor 488 conjugated secondary antibody. CD68 positive cells emitting bright green were detected as tissue macrophages. The synovial tissues were also treated with anti-Sulf-1 and anti-Sulf-2 primary antibodies, and Alexa Fluor 546 conjugated secondary antibody demonstrated Sulf-1 and Sulf-2 emitting orange to red under fluorescence microscopy. Hoechst 33258 was used for counterstaining the nuclei to indicate the cellular expression of the molecules in the synovium.

Sulf-1 and Sulf-2 positive cells were widely distributed throughout the lining and sublining layers of inflamed RA synovium, which included FLSs and macrophages. Merged images revealed synovial macrophages colored bright yellow as a result of dual positive staining of CD68 and Sulfs. Macrophages were densely infiltrated in the sublining layer (Fig. 1B, upper panel). However, lymphoid follicles composed of small lymphocytes were completely negative for both CD68 and Sulfs (Fig. 1B, lower panel).

The results demonstrate that circulating and tissue lymphocytes or monocytes are not reactive for Sulf expression in response to proinflammatory stimuli and inflammation. In contrast, macrophages stimulated and differentiated in inflamed tissues showed active upregulation of Sulf-1 and Sulf-2.

Based on the Sulf expression of inflammatory cells, this study aimed to evaluate the roles of macrophage Sulf-1 and Sulf-2 in inflammation. Macrophages are the prime scavenger and antigen-presenting cells (APC) that participate in the initiation of inflammatory and immune responses. Sulfs-related functional changes in macrophages were analyzed using an antigen presentation assay with the regulation of Sulf expression in murine peritoneal macrophages.

Sulf expression in macrophages was knocked down using siRNAs against Sulf-1 and Sulf-2 and upregulated by transfection with pcDNA3.1/Sulf-1 and pcDNA3.1/Sulf-2 plasmids. Downregulation of Sulf gene transcription by Sulf-1 and Sulf-2 siRNAs in mac-
**Fig. 1.** Sulfatase (Sulf) expression in the inflammatory cells with proinflammatory stimuli and tissue inflammation. Murine peritoneal macrophages show increased expression of Sulf-1 and Sulf-2 upon stimulation with interleukin (IL)-1β. (A) However, normal non-treated (NT) peripheral blood mononuclear cells (PBMCs) show weak expression of Sulfs and no change in Sulf expression by *in vitro* activation with phytohemagglutinin and lipopolysaccharides (LPS) (SW1353, a cell line, as positive control) (two-colored double immunofluorescence stain, x200). (B) Rheumatoid arthritis (RA) synovial tissues show chronic inflammatory reactions. Synovial macrophages as CD68 positive cells infiltrated in sublining layers. Lymphocytes as the CD68 negative cells of small nuclei clustered in lymphoid follicles. (B, upper panel) Two-colored double immunofluorescence staining of Sulfs and CD68 revealed synovial macrophages colored bright yellow as the result of dual positive staining of CD68 and Sulfs. (B, lower panel) However, lymphocytes are completely negative for both CD68 and Sulfs (two-colored double immunofluorescence stain, x200). MCP-1, monocyte chemotactic protein-1.
Macrophage sulfatase (Sulf) expression was knocked down using small interfering (si) RNAs against Sulf-1 and Sulf-2, and up-regulated by transfection with pcDNA3.1/Sulf-1 and pcDNA3.1/Sulf-2 plasmids. (A) Downregulation of Sulf gene transcription in macrophages transfected with Sulf-1 and Sulf-2 siRNAs, as compared with non-treated (NT) and siRNAs controls, was confirmed by real-time polymerase chain reaction. (B) Overexpression of Sulf-1 and Sulf-2 in macrophages transfected with pcDNA3.1/Sulf-1 and Sulf-2 plasmids, as compared with those of NT and pcDNA3.1 controls, was confirmed by western blot. **p<0.01.

Antigen presentation by macrophages involves a wide range of responses initiated by pattern recognition and phagocytosis. Therefore, macrophage phagocytic activity was assessed by regulating Sulf-1 and Sulf-2 expression in the same manner as that in the antigen presentation assay. Murine peritoneal macrophages were treated with siRNAs or pcDNA3.1 plasmids to suppress or enhance Sulf expression, respectively (Fig. 2).

Macrophages were cultured with zymosan, and the number of zymosan granules engulfed by macrophages was compared using microscopic images. Sulfs-knockdown macrophages showed marked suppression of zymosan phagocytosis compared to controls (Fig. 4A, upper panel). Macrophages overexpressing Sulfs showed brisk phagocytic activity against zymosan (Fig. 4A, lower panel), which was completely opposite to the Sulfs-knockdown macrophages. In addition to the microscopic images, phagocytosis of zymosan by macrophages was analyzed quantitatively by measuring the absorbance at 450 nm using spectrophotometry. The absorbance of Sulfs-knockdown macrophages was significantly lower than that of controls (zymosan+control and zymosan+siControl, respectively) (control: 1.28 ± 0.04 absorbance unit [AU], zymosan+cytochalasin D: 1.49 ± 0.03 AU, zymosan+control: 2.23 ± 0.05 AU, zymosan+siControl: 2.07 ± 0.03 AU, zymosan+Sulf-1 siRNA: 1.6 ± 0.1 AU, zymosan+Sulf-2 siRNA: 1.74 ± 0.09 AU; values are mean ± SD, respectively). Inversely, Sulfs-overexpressing macrophages showed higher absorbance compared with controls (zymosan+control and zymosan+pcDNA3.1, respectively) (control: 1.28 ± 0.04 AU, zymosan+cytochalasin D: 1.49 ± 0.03 AU, zymosan+control: 2.23 ± 0.05 AU, zymosan+siControl: 2.07 ± 0.03 AU, zymosan+Sulf-1 siRNA: 1.6 ± 0.1 AU, zymosan+Sulf-2 siRNA: 1.74 ± 0.09 AU; values are mean ± SD, respectively). The effects of Sulfs on macrophage phagocyto-
Fig. 3. Antigen presentation assay with modification of sulfatase (Sulf) expression. Murine peritoneal macrophages were cultured with ovalbumin. (A) Macrophages with Sulf-1 or Sulf-2 suppression show significantly lower \( ^{3} \text{H} \)-thymidine radioactivity incorporated into interleukin-2 dependent CTLL-2 cells as compared with controls. (B) Macrophages with upregulation of Sulf-1 or Sulf-2 show significantly higher \( ^{3} \text{H} \)-thymidine radioactivity compared with controls. The \( ^{3} \text{H} \)-thymidine radioactivity is reciprocal by Sulf suppression and overexpression. The changes in radioactivity are more prominent with Sulf-1 regulation than with Sulf-2. cpm, counts per minute. **p<0.01.

Discussion

HSPGs are extracellular matrix glycoproteins modified with specific HS polymers [18]. HS is a linear polysaccharide of a variably sulfated repeating disaccharide (GAG). Covalently linked to various core proteins, one or more HS chains comprise extraordinarily heterogeneous structures of HSPGs. The cell-surface HSPG molecules are classified into four groups; syndecans, glypicans, betaglycans, and CD44 family proteins. In addition, the sulfation pattern and level of HSPGs at four different sites (N-, 3-O, and 6-O of glucosamine and 2-O of uronic acid) give rise to molecular diversity [19].

Regarding HSPGs related to infection/inflammation, HSPG is rarely expressed in normal resting leukocytes [20]. However, as monocytes undergo progressive differentiation into tissue macrophages during the recruitment process, activated human macrophages express syndecan-2 on the cell surface [2]. HSPGs are known to facilitate morphogen gradients that are essential for cell development and chemokine gradients for leukocyte recruitment and homing [21,22]. In addition to the presence of HSPGs in inflammatory cells, studies on *Chlamydia muridarum* have demonstrated that the level of HSPG 6-O sulfation is a critical determinant of infection, and that 6-O endosulfatases are involved in microbial pathogenesis as modulators [23].

The sulfation pattern and level of variably sulfated molecules may be the major determinants of the biological activities of HSPGs. Regarding posttranslational modulation, the extracellular 6-O-endosulfatases, Sulf-1 and Sulf-2, desulfate HSPGs in the extracellular compartment, in contrast to the biosynthesis and sulfation at the Golgi apparatus [24].

Aberrant expression and activity of Sulf-1 and Sulf-2 have been linked to diseases such as cancers [25-28] and developmental changes [29-31]. However, little information is available concerning their role in inflammation. Sulf-1 and Sulf-2 expression were reported to be upregulated in articular cartilages and synovial tissues of human osteoarthritis (OA) and murine OA knees [32]. These findings might indicate an association between Sulfs and the reactive synovium. Although no information is available about Sulf-1 and Sulf-2 in inflammatory tissues, the current study shows that synoviocytes and synovial macrophages upregulate Sulf-1 and Sulf-2 expression in the inflamed synovium of RA.

In this study, Sulf-1 and Sulf-2 were analyzed with regard to their
expression in inflammatory cells of peripheral blood and tissues, and their functions in inflammation.

The results demonstrated that macrophages expressed Sulf-1 and Sulf-2. In addition, Suls are upregulated in macrophages in response to proinflammatory stimuli and tissue inflammation, but not in lymphocytes or monocytes.

Infiltrated macrophages are the core features of inflammation, in which they play roles in eliminating infection, clearing up debris, and restoring tissue homeostasis as an effector of innate immunity [33]. In addition, macrophages, as professional APCs, are involved in the induction of acquired immunity that requires coordinated interplay between innate immune cells and naive lymphocytes.

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The capacity to take up and process antigens, express class II major histocompatibility complex (MHC) glycoproteins on their surface, and synthesize and release IL-1 are indispensable for the cells to function as APCs [34].

T cell stimulation by antigen presentation depends on the efficiency of antigen capture by APCs [35], and HSPGs behave as receptors or co-receptors in MHC class II-restricted antigen presentation of proteins [36]. Earlier studies have reported that a critical level of HSPG ligation is necessary and sufficient to trigger phagocytic uptake into epithelial cells [37]. Therefore, in the context of HSPGs and their sulfation as key determinants of biological activities, Sulfs may function as regulators of T cell activation and immunity by modulating antigen presentation and phagocytosis by APCs.

This study demonstrates that antigen presentation activity by macrophages is strongly correlated with Sulfs expression, suppressed in Sulf-1 and Sulf-2 knockdown macrophages, and enhanced in Sulfs-overexpressing macrophages.

For the processing and presentation of phagocytosed antigenic substrates to lymphocytes, exogenous antigen uptake into professional APCs is initiated by some types of endocytic mechanisms: receptor-mediated endocytosis through the clathrin-coated pit system [38], pinocytosis, particularly macropinocytosis as a consequence of membrane ruffling [39] and phagocytosis [40].

In relation to the Sulfs-dependent antigen presentation, the current study evaluated macrophage phagocytosis and showed that the phagocytic activity of macrophages also had a strong correlation with Sulfs expression, enhanced in Sulfs-1 and Sulfs-2 overexpressing macrophages, and vice versa. This is entirely concordant with the results of the antigen presentation assay of macrophages to T lymphocytes. However, in this study, the protein expression levels were not determined after Sulf-1 and Sulf-2 gene suppression was confirmed by RT-PCR, which could be a limitation of the assay.

Taken together, these observations clearly demonstrate that Sulf-1 and Sulf-2 expression is actively regulated by macrophages in correlation with tissue inflammation, and that Sulfs, particularly Sulf-1, may play a role in facilitating inflammation through macrophage phagocytosis and antigen presentation. To the best of our knowledge, this is the first report on Sulfs and macrophage activity.

Notes

Ethical statements

The study protocol was approved by the Institutional Review Board (IRB) of Yeungnam University Hospital (IRB No: YUH-12-0305-O2). Informed consent was obtained from participants, which was confirmed by the IRB. This study was conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Conflicts of interest

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

Author contributions

Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Visualization, Investigation, Resources, Validation: all authors; Software, Supervision: HSK, YHH; Writing-original draft: all authors; Writing-review & editing: all authors.

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References


Prognostic impact of chromogranin A in patients with acute heart failure

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Background: Chromogranin A (CgA) levels have been reported to predict mortality in patients with heart failure. However, information on the prognostic value and clinical availability of CgA is limited. We compared the prognostic value of CgA to that of previously proven natriuretic peptide biomarkers in patients with acute heart failure.

Methods: We retrospectively evaluated 272 patients (mean age, 68.5±15.6 years; 62.9% male) who underwent CgA test in the acute stage of heart failure hospitalization between June 2017 and June 2018. The median follow-up period was 348 days. Prognosis was assessed using the composite events of 1-year death and heart failure hospitalization.

Results: In-hospital mortality rate during index admission was 7.0% (n = 19). During the 1-year follow-up, a composite event rate was observed in 12.1% (n = 33) of the patients. The areas under the receiver-operating characteristic curves for predicting 1-year adverse events were 0.737 and 0.697 for N-terminal pro-B-type natriuretic peptide (NT-proBNP) and CgA, respectively. During follow-up, patients with high CgA levels (> 158 pmol/L) had worse outcomes than those with low CgA levels (≤ 158 pmol/L) (85.2% vs. 58.6%, p < 0.001). When stratifying the patients into four subgroups based on CgA and NT-proBNP levels, patients with high NT-proBNP and high CgA had the worst outcome. CgA had an incremental prognostic value when added to the combination of NT-proBNP and clinically relevant risk factors.

Conclusion: The prognostic power of CgA was comparable to that of NT-proBNP in patients with acute heart failure. The combination of CgA and NT-proBNP can improve prognosis prediction in these patients.

Keywords: Biomarkers; Chromogranin A; Heart failure; Prognosis

Introduction

Heart failure (HF) is an increasing health problem worldwide [1-3]. Despite recent progress in pharmacologic and implantable cardiac device treatment, the mortality of patients with HF remains substantially high [2-6]. There is increasing interest in the use of many biological markers, including natriuretic peptides, to more accurately determine patient prognosis, such as mortality and HF hospitalization.

Neurohormonal deterioration plays a key role in the development of HF. Numerous biomarkers and therapeutic approaches have been established to elucidate this pathophysiological mecha-
nism. Natriuretic peptide has become a reliable biomarker for the diagnosis and estimation of prognosis in patients with HF. Additionally, the use of novel biomarkers may guarantee a more accurate estimation of the prognosis of HF and can be valuable for understanding the pathophysiology of HF. Chromogranin A (CgA) is a prohormone produced in many tissues, including neuroendocrine and myocardial tissues. The level of CgA is closely correlated with sympathetic activity in the adrenal gland and peripheral nervous system, suggesting that it might be a marker of sympathetic activity in humans [7]. In addition, it has been known as a biomarker for neuroendocrine tumors [8] and has recently been found to be associated with prognosis in patients with cardiovascular disease [9-12]. Recent studies have shown that CgA is an independent predictor of long-term mortality and HF hospitalization in patients with acute coronary syndromes [9]. In patients with HF, circulating CgA levels are associated with functional status, HF hospitalization, and mortality [10].

The present study aimed to investigate (1) the prognostic value of CgA in patients with acute HF and (2) the additive prognostic impact of CgA when measured together with N-terminal pro-B-type natriuretic peptide (NT-proBNP) in patients with acute HF.

Materials and methods

1. Study population

We retrospectively evaluated patients who underwent CgA testing during the acute stage of HF hospitalization between June 2017 and June 2018. A total of 272 patients were enrolled, and the median follow-up duration was 348 days.

The diagnosis of HF was established based on current guidelines, taking into account symptoms, laboratory findings, and radiographic and echocardiographic findings.

Data on demographic characteristics, medications, laboratory tests, and prognosis were collected from the patients’ medical charts. Initial transthoracic echocardiographic findings were collected from the images and reports from the database. NT-proBNP (The Dimension Vista PBNP Flex reagent cartridge; Erlangen, Germany) and CgA (Cisbio CgA ELISA kit; Codolet, France) were tested in venous blood and within 24 hours of hospital admission. Patients were followed up for 12 months after the index hospitalization. The primary endpoint was a composite event of 1-year death and hospitalization for HF. The death certificates and National Health Insurance data were reviewed to determine survival or death.

2. Statistics

Data are presented as percentages for baseline characteristics and categorical variables, and comparisons between them were assessed using the chi-square test. The distribution of continuous variables is expressed as the mean ± standard deviation or median (interquartile range), and the differences were compared using the independent t-test.

A receiver-operating characteristic (ROC) curve was used to identify the optimal cutoff points for the CgA and NT-proBNP levels to determine the potential relationship between CgA and prognosis. Delong’s method was used to compare the area under the curves (AUCs) between CgA and NT-proBNP. All patients were classified into four groups according to the cutoff levels for NT-proBNP and CgA obtained from the ROC curve analysis. Kaplan-Meier survival analysis was performed based on these four groups. Bonferroni correction was used in the subgroup analysis that tested the prognostic value of CgA in patients with high and low NT-proBNP levels. All analyses were two-tailed, and p-values of < 0.05 were considered statistically significant.

The relationship between the two biomarkers and prognosis (composite outcome of 1-year death and hospitalization for HF) of the patients were investigated.

Results

1. Baseline characteristics

Patients who underwent CgA and NT-proBNP testing between June 2017 and June 2018 in the acute stage of HF hospitalization were enrolled retrospectively. A total of 272 patients (mean age, 68.5 ± 15.6 years; 62.9% male) were enrolled, and the median follow-up duration was 348 days. Nearly half of the patients had New York Heart Association (NYHA) class III–IV symptoms (45.2%). Of the total patients, 133 (48.9%) had hypertension, 86 (31.6%) had diabetes mellitus, 54 (19.9%) had atrial fibrillation, and 108 (39.7%) had ischemic heart disease. The mean left ventricular ejection fraction (LVEF) was 42.6% ± 16.1%, the mean NT-proBNP level was 10,650.1 ± 26,302.4 pg/mL, and the mean CgA level was 279.8 ± 392.6 pmol/L (Table 1). The CgA level increased with a decrease in LVEF and an increase in NYHA class (Supplementary Figs. 1, 2).

Patients in both the high NT-proBNP and high CgA groups were more likely to be older and to be prescribed a loop diuretic in their discharge medications than those in the low NT-proBNP and low CgA groups. The high CgA group had higher NT-proBNP levels than the low CgA group. In addition, higher CgA levels were observed in the high NT-proBNP group than in the low NT-proBNP group. In the high CgA group, de novo HF was observed less frequently than in the low CgA group. Atrial fibrillation, ischemic heart disease, medication before admission, and estimated glomer-
ular filtration rate were not different between the two groups regardless of NT-proBNP and CgA levels (Table 1, Supplementary Table 1).

2. Prognostic value of serum CgA and NT-proBNP

In-hospital mortality rate of the study population was 7.0% (n = 19). During the follow-up period, the composite event of 1-year death and hospitalization for HF was observed in 12.1% of patients (n = 33). In the ROC analysis to predict the composite event of 1-year death and hospitalization, the AUCs were 0.737 and 0.697 for NT-proBNP and CgA, respectively. The cutoff values for predicting the prognosis for CgA and NT-proBNP were 158 pmol/L and 3,429 pg/mL, respectively (Fig. 1). During the follow-up, patients with high CgA (> 158 pmol/L) were more likely to have worse outcomes (85.2% vs. 58.6%, p < 0.001) in the Kaplan-Meier analysis. Age, NYHA class, atrial fibrillation, de novo HF, NT-proBNP level, and CgA level were significantly associated with prognosis in the univariate analysis. In the multivariable analysis model, CgA was an independent factor associated with 1-year death and hospitalization for HF (hazard ratio [HR], 1.059/100 pmol/L; 95% confidence interval [CI], 1.007–1.115, p = 0.027), as was age, NYHA class, and de novo HF (Table 2).

Based on the cutoff values for CgA (158 pmol/L) and NT-proBNP (3,429 pg/mL), the study cohort was divided into four groups: low NT-proBNP and low CgA (n = 106), low NT-proBNP and high CgA (n = 40), high NT-proBNP and low CgA (n = 60), and high NT-proBNP and high CgA (n = 66). The Kaplan-Meier survival analysis showed that the group with low levels of both

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### Table 1. Baseline characteristics of the patients according to level of serum NT-proBNP and CgA

<table>
<thead>
<tr>
<th>Variable</th>
<th>All (n = 272)</th>
<th>NT-proBNP</th>
<th>CgA</th>
<th>p-value</th>
<th>Low (n = 146)</th>
<th>High (n = 126)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low (n = 166)</td>
<td>High (n = 106)</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>68.5 ± 15.6</td>
<td>66.7 ± 15.5</td>
<td>70.5 ± 15.5</td>
<td>0.040</td>
<td>65.7 ± 16.6</td>
<td>72.8 ± 12.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Male sex</td>
<td>171 (62.9)</td>
<td>92 (63.0)</td>
<td>79 (62.7)</td>
<td>&gt; 0.999</td>
<td>110 (66.3)</td>
<td>61 (57.5)</td>
<td>0.186</td>
</tr>
<tr>
<td>Hypertension</td>
<td>133 (48.9)</td>
<td>64 (43.8)</td>
<td>69 (54.8)</td>
<td>0.094</td>
<td>61 (36.7)</td>
<td>72 (67.9)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>86 (31.6)</td>
<td>39 (26.7)</td>
<td>47 (37.3)</td>
<td>0.081</td>
<td>37 (22.3)</td>
<td>49 (46.2)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>54 (19.9)</td>
<td>27 (18.5)</td>
<td>27 (21.4)</td>
<td>0.651</td>
<td>32 (19.3)</td>
<td>22 (20.8)</td>
<td>0.887</td>
</tr>
<tr>
<td>De novo heart failure</td>
<td>204 (75.0)</td>
<td>119 (81.5)</td>
<td>85 (67.5)</td>
<td>0.011</td>
<td>132 (79.5)</td>
<td>72 (67.9)</td>
<td>0.044</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>149 (54.8)</td>
<td>106 (72.6)</td>
<td>43 (34.1)</td>
<td></td>
<td>94 (56.6)</td>
<td>55 (51.9)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>65 (23.9)</td>
<td>23 (15.8)</td>
<td>42 (33.3)</td>
<td></td>
<td>42 (25.3)</td>
<td>23 (21.7)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>58 (21.3)</td>
<td>17 (11.6)</td>
<td>41 (32.5)</td>
<td></td>
<td>30 (18.1)</td>
<td>28 (26.4)</td>
<td></td>
</tr>
<tr>
<td>Ischemic etiology</td>
<td>108 (39.7)</td>
<td>59 (40.4)</td>
<td>49 (38.9)</td>
<td>0.859</td>
<td>64 (38.6)</td>
<td>44 (41.9)</td>
<td>0.673</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>135.5 ± 30.2</td>
<td>133.1 ± 28.3</td>
<td>138.3 ± 32.2</td>
<td>0.155</td>
<td>132.2 ± 28.1</td>
<td>140.6 ± 32.8</td>
<td>0.024</td>
</tr>
<tr>
<td>Heart rate (beat/min)</td>
<td>88.6 ± 22.5</td>
<td>84.0 ± 20.4</td>
<td>94.0 ± 23.7</td>
<td>&lt; 0.001</td>
<td>86.8 ± 21.0</td>
<td>91.4 ± 24.5</td>
<td>0.101</td>
</tr>
<tr>
<td><strong>Laboratory findings</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Low (n = 166)</td>
<td>High (n = 106)</td>
<td>p-value</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>12.6 ± 2.1</td>
<td>12.4 ± 2.3</td>
<td>12.8 ± 2.0</td>
<td>0.126</td>
<td>12.5 ± 2.2</td>
<td>12.8 ± 2.0</td>
<td>0.404</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>2.0 ± 9.4</td>
<td>2.5 ± 12.8</td>
<td>3.0 ± 1.0</td>
<td>0.244</td>
<td>2.3 ± 11.9</td>
<td>1.4 ± 2.1</td>
<td>0.337</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73 m³)</td>
<td>78.0 ± 191.9</td>
<td>87.5 ± 260.7</td>
<td>67.0 ± 31.6</td>
<td>0.351</td>
<td>64.5 ± 30.3</td>
<td>99.1 ± 304.5</td>
<td>0.248</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>137.3 ± 6.0</td>
<td>137.3 ± 6.0</td>
<td>137.4 ± 6.0</td>
<td>0.913</td>
<td>137.6 ± 6.2</td>
<td>137.0 ± 5.7</td>
<td>0.405</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>4.3 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>4.3 ± 0.6</td>
<td>0.459</td>
<td>4.3 ± 0.6</td>
<td>4.2 ± 0.6</td>
<td>0.354</td>
</tr>
<tr>
<td>CgA (pmol/L)</td>
<td>279.8 ± 392.6</td>
<td>160.1 ± 222.2</td>
<td>418.5 ± 490.8</td>
<td>&lt; 0.001</td>
<td>81.2 ± 38.7</td>
<td>590.9 ± 485.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>NT-proBNP (pg/mL)</td>
<td>10,650.1 ± 26,302.4</td>
<td>1,204.1 ± 1,024.7</td>
<td>21,595.4 ± 35,688.4</td>
<td>&lt; 0.001</td>
<td>4,336.4 ± 6,456.9</td>
<td>20,537.6 ± 39,475.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td></td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>≥ 50</td>
<td>114 (41.9)</td>
<td>86 (58.9)</td>
<td>28 (22.2)</td>
<td></td>
<td>79 (47.6)</td>
<td>35 (33.0)</td>
<td></td>
</tr>
<tr>
<td>40–49</td>
<td>43 (15.8)</td>
<td>27 (18.5)</td>
<td>16 (12.7)</td>
<td></td>
<td>23 (13.9)</td>
<td>20 (18.9)</td>
<td></td>
</tr>
<tr>
<td>&lt; 40</td>
<td>115 (42.3)</td>
<td>33 (22.6)</td>
<td>82 (65.1)</td>
<td></td>
<td>64 (38.6)</td>
<td>51 (48.1)</td>
<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>42.6 ± 16.1</td>
<td>48.9 ± 14.0</td>
<td>35.4 ± 15.5</td>
<td>&lt; 0.001</td>
<td>43.7 ± 16.7</td>
<td>41.0 ± 15.1</td>
<td>0.178</td>
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<tr>
<td>Renal replacement therapy</td>
<td>7 (2.6)</td>
<td>0 (0)</td>
<td>7 (5.6)</td>
<td>0.012</td>
<td>1 (0.6)</td>
<td>6 (5.7)</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Values are presented as mean±standard deviation or number (%).

NT-proBNP, N-terminal pro-B-type natriuretic peptide; CgA, chromogranin A; NYHA, New York Heart Association; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction.
NT-proBNP and CgA had the best 1-year survival rate (88.9%), followed by the group with low NT-proBNP and high CgA levels (86.6%), the group with high NT-proBNP and low CgA levels (78.6%), and the group with both high NT-proBNP and CgA levels (40.2%, \( p < 0.001 \)) (Fig. 2). In the subgroup of patients with high NT-proBNP levels, low CgA levels were associated with patient prognosis (40.2% vs. 78.6%, \( p < 0.001 \)), whereas there was no significant prognostic value in the subgroup of patients with low NT-proBNP levels (86.6% vs. 88.9%, \( p > 0.999 \)).

To investigate the predictive value of CgA, we created three models: (1) the baseline model including the risk factors of age, sex, NYHA class, atrial fibrillation, and de novo HF; (2) baseline model+NT-proBNP; and (3) baseline model+NT-proBNP+CgA. The addition of serum NT-proBNP and CgA to the established risk factors increased the provided incremental information for predicting the prognosis (global chi-square 42.4 to 47.2, \( p = 0.030 \)) (Fig. 3).

**Discussion**

In the present study, blood CgA levels in patients with acute HF were found to be an independent factor associated with 1-year adverse events (global chi-square from 47.2 to 51.4, \( p = 0.039 \)) (Fig. 3).

**Table 2.** Univariable and multivariable analysis for prediction of composite event of 1-year death and hospitalization for heart failure

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate model HR (95% CI)</th>
<th>( p )-value</th>
<th>Multivariate model HR (95% CI)</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.038 (1.016–1.059)</td>
<td>&lt; 0.001</td>
<td>1.033 (1.010–1.056)</td>
<td>0.005</td>
</tr>
<tr>
<td>Male sex</td>
<td>0.962 (0.570–1.622)</td>
<td>0.909</td>
<td>1.343 (0.771–2.340)</td>
<td>0.298</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.194 (0.723–1.974)</td>
<td>0.488</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>1.267 (0.751–2.137)</td>
<td>0.375</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>2.271 (1.330–3.877)</td>
<td>0.003</td>
<td></td>
<td></td>
</tr>
<tr>
<td>De novo onset</td>
<td>0.329 (0.199–0.544)</td>
<td>&lt; 0.001</td>
<td>1.784 (1.029–3.093)</td>
<td>0.394</td>
</tr>
<tr>
<td>NYHA class III (reference, NYHA class II)</td>
<td>1.735 (0.932–3.230)</td>
<td>0.082</td>
<td>0.439 (0.259–0.744)</td>
<td>0.002</td>
</tr>
<tr>
<td>NYHA class IV (reference, NYHA class II)</td>
<td>2.861 (1.578–5.187)</td>
<td>0.001</td>
<td>1.225 (0.636–2.362)</td>
<td>0.543</td>
</tr>
<tr>
<td>Ischemic etiology</td>
<td>0.989 (0.608–1.680)</td>
<td>0.966</td>
<td>1.980 (1.051–3.729)</td>
<td>0.035</td>
</tr>
<tr>
<td>SBP (/10 mmHg)</td>
<td>1.010 (0.932–1.095)</td>
<td>0.804</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart rate (/10 beats/minute)</td>
<td>1.111 (0.993–1.244)</td>
<td>0.066</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>1.000 (0.882–1.131)</td>
<td>0.984</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR (/10 mL/min/1.73 m(^2))</td>
<td>1.012 (0.930–1.050)</td>
<td>0.696</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal replacement therapy</td>
<td>2.573 (0.933–7.10)</td>
<td>0.068</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium (/10 mEq/L)</td>
<td>1.035 (0.693–1.544)</td>
<td>0.688</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Potassium (/10 mEq/L)</td>
<td>0.660 (0.011–40.850)</td>
<td>0.844</td>
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<td></td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>0.996 (0.980–1.012)</td>
<td>0.590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NT-proBNP (/10,000 pg/mL)</td>
<td>1.096 (1.047–1.147)</td>
<td>&lt; 0.001</td>
<td>1.051 (0.994–1.111)</td>
<td>0.081</td>
</tr>
<tr>
<td>CgA (/100 pmol/L)</td>
<td>1.096 (1.050–1.145)</td>
<td>&lt; 0.001</td>
<td>1.059 (1.007–1.115)</td>
<td>0.027</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval; NYHA, New York Heart Association; SBP, systolic blood pressure; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; CgA, chromogranin A.

https://doi.org/10.12701/yujm.2020.00843
death and hospitalization for HF. We divided the study cohort into four groups based on NT-proBNP and CgA blood concentrations. Those with low NT-proBNP and low CgA levels showed the lowest rates of 1-year death and hospitalization for HF. High NT-proBNP and high CgA levels were associated with the worst outcomes. Our findings demonstrate the advantage of adding CgA levels to a well-established biomarker, NT-proBNP, for better risk stratification. Simultaneous measurement of NT-proBNP and CgA can help predict death in patients with acute HF more accurately than the estimation of NT-proBNP alone. In particular, CgA has a low significance as a prognostic predictor of 1-year death and HF hospitalization in patients with low NT-proBNP levels and may be more helpful as a prognostic predictor in patients with high NT-proBNP levels. In our study, the high NT-proBNP group was older and consisted of a greater proportion of individuals with NYHA class III–IV, suggesting that CgA may be more helpful in predicting the prognosis of high-risk groups of patients with acute HF.

Ceconi et al. [10] suggested that CgA levels were increased in patients with chronic HF and were a predictor of mortality. Røsjø et al. [13] investigated the prognostic value of CgA in patients with stabilized HF from the GISSI-Heart Failure trial. In the univariable analysis, increased CgA plasma concentrations were associated with all-cause mortality, with hazard ratios between 1.58 (95% CI, 1.17–2.11) and 2.35 (95% CI, 1.78–3.10). However, after adjustment for clinically relevant risk factors, the association was not significant.

A recent study investigated the underlying mechanism of CgA increase in patients with acute HF, suggesting a role for CgA in patients with acute HF [11]. CgA is a member of the granin protein family, which plays a role in the endocrine and paracrine system and seems to play a role in cardiovascular disease.

![Composite event of death and hospitalization for heart failure in 360 days](https://doi.org/10.12701/yujm.2020.00843)

Fig. 2. Kaplan-Meier survival analysis of the groups according to the levels of chromogranin A (CgA; cutoff value=158 pmol/L) and N-terminal pro-B-type natriuretic peptide (NT-proBNP; cutoff value=3,429 pg/mL).
mechanisms of increased CgA in patients with HF is thought to be the impaired processing of CgA to CgA fragment catestatin [11]. Ottesen et al. [11] revealed that the fragment catestatin may directly dysregulate cardiomyocyte Ca\(^{2+}\) handling, indicating that elevated CgA can be related to patient prognosis in the acute stage of HF.

NT-proBNP is a well-established biomarker in a variety of heart diseases, including HF, because of its correlation with clinical status, echocardiographic parameters, laboratory findings, and short- and long-term prognosis [14-16]. NT-proBNP is a neurohormone released by the ventricles of the heart as a result of volume or pressure overload and has a different mechanism than CgA secretion. Our study indicates that when biomarkers from two different mechanisms are combined, they are synergistic in predicting the long-term prognosis of patients with acute HF. The simultaneous application of CgA and NT-proBNP allows a more accurate estimate of the prognosis of patients with acute HF in clinical practice.

Our study had some limitations to consider. First, this was a relatively small retrospective study conducted in a single center. Thus, the results of the present study need to be validated in larger trials. Second, although we included all patients available during the index period, there may have been some selection bias in the study population.

CgA presented prognostic power comparable to that of the proven biomarker NT-proBNP in patients with acute HF. CgA combined with NT-proBNP levels demonstrated an additive prognostic value in these patients.

Supplementary materials
Supplementary materials can be found via https://doi.org/10.12701/yujm.2020.00843

Notes

Ethical statements
The Institutional Review Board (IRB) of Kyungpook National University Hospital approved this study and permitted the review and publication of patient records (IRB No: 2019-12-017-001). All data were anonymized before analysis, and the need for written informed consent was waived by the IRB due to the retrospective nature of this study.

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

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Author contributions
Conceptualization: DHY, BEP, YJP, HJK, SYJ, MHB, JHL, HSP, YC, SCC; Investigation: BEP, YJP, HJK; Data curation: HNK, DHY, BEP, YJP, HJK; Formal analysis: HNK, DHY, SYJ, MHB, HSP, YC, SCC; Funding acquisition, Validation: DHY, SYJ, JHL, HSP, YC, SCC; Methodology: DHY, SYJ, MHB, JHL, YC; Project administration: DHY; Supervision: DHY, SYJ, MHB, HSP, YC, SCC; Writing-original draft: HNK, YJP; Writing-review & editing: HNK, DHY, BEP, SYJ, MHB, JHL, HSP.

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References


Background: Cancer patients have been disproportionately affected by the coronavirus disease 2019 (COVID-19) pandemic, with high rates of severe outcomes and mortality. Fever is the most common symptom in COVID-19 patients. During the COVID-19 pandemic, physicians may have difficulty in determining the cause of fever (COVID-19, another infection, or cancer fever) in cancer patients. Furthermore, there are no specific guidelines for managing cancer patients with fever during the COVID-19 pandemic. Thus, this study evaluated the clinical characteristics and outcomes of cancer patients with fever during the COVID-19 pandemic.

Methods: This study retrospectively reviewed the medical records of 328 cancer patients with COVID-19 symptoms (fever) admitted to five hospitals in Daegu, Korea from January to October 2020. We obtained data on demographics, clinical manifestations, laboratory test results, chest computed tomography images, cancer history, cancer treatment, and outcomes of all enrolled patients from electronic medical records.

Results: The most common COVID-19-like symptoms were fever (n = 256, 78%). Among 256 patients with fever, only three (1.2%) were diagnosed with COVID-19. Most patients (253, 98.8%) with fever were not diagnosed with COVID-19. The most common solid malignancies were lung cancer (65, 19.8%) and hepatobiliary cancer (61, 18.6%). Twenty patients with fever experienced a delay in receiving cancer treatment. Eighteen patients discontinued active cancer treatment because of fever. Major events during the treatment delay period included death (2.7%), cancer progression (1.5%), and major organ dysfunction (2.7%).

Conclusion: Considering that only 0.9% of patients tested for COVID-19 were positive, screening for COVID-19 in cancer patients with fever should be based on the physician’s clinical decision, and patients might not be routinely tested.

Keywords: COVID-19; Fever; Neoplasms; Neoplastic fever
Introduction

Since its emergence in Wuhan, China in December 2019, coronavirus disease 2019 (COVID-19) has become a pandemic [1]. Patients with cancer are susceptible to infection during the pandemic due to the effect of anticancer treatment and the immunosuppressive properties of cancer itself [2]. A Chinese study reported that approximately 1% of patients with COVID-19 had cancer, which was five-fold higher than the general cancer incidence in China [3]. Onder et al. [4] reported that approximately 20% of people who died of COVID-19 had a history of active cancer in the past 5 years. As a group at high risk of infection during the COVID-19 pandemic, physicians should pay more attention to cancer patients.

The clinical symptoms of COVID-19 include fever, cough, dyspnea, fatigue, headache, sore throat, myalgia, and diarrhea [5,6]. Fever is the most common symptom of COVID-19, and many patients are tested for COVID-19 due to fever [7-9]. In cancer patients, fever might be the only sign of infection, which can be life-threatening in those undergoing chemotherapy. There are other causes of fever in cancer patients besides infection. Neoplastic fever, a fever caused by cancer itself, has been reported as the most common cause of fever of unknown origin in cancer patients [10]. Since the emergence of the COVID-19 pandemic, many cancer patients have visited outpatient clinics or emergency units because of fever, and most of them have been tested for COVID-19 regardless of the possible causes of fever, including other infections and neoplastic fever. In addition, physicians sometimes face difficulty in determining the cause of the fever (COVID-19 vs. others) in cancer patients with COVID-19-like symptoms. Thus, we analyzed the clinical characteristics and outcomes of 328 patients with cancer who had fever and/or other COVID-19-like symptoms during the COVID-19 pandemic at five hospitals in Daegu.

Materials and methods

1. Patient selection

This study retrospectively reviewed the medical records of 328 cancer patients with COVID-19-like symptoms who had been tested for COVID-19 at five hospitals (Kyungpook National University Chilgok Hospital, Yeungnam University Medical Center, Daegu Fatima Hospital, Daegu Catholic University Medical Center, and Keimyung University Dongsan Medical Center) in Daegu from January to October 2020. COVID-19-like symptoms included fever, cough, sore throat, rhinorrhea, myalgia, and arthritis. Fever was defined as a tympanic temperature of at least 38°C (100.4°F) [11]. All patients were tested for COVID-19. COVID-19 was diagnosed based on the criteria published by the World Health Organization and confirmed by real-time polymerase chain reaction (PCR) assays of nasal and/or pharyngeal specimens [12-14]. COVID-19 death was defined as death resulting from a clinically compatible illness in a patient with confirmed COVID-19 unless a clear alternative cause of death that cannot be associated with COVID-19 could be proven.

2. Data collection

We obtained data on demographics, clinical manifestations, laboratory test results, chest computed tomography images, cancer history, cancer treatment, and outcomes of all enrolled patients from electronic medical records. Patient data were collected retrospectively using uniform database templates to ensure consistent data collection.

3. Statistical analysis

Statistical analysis was performed using IBM SPSS version 20.0 (IBM Corp, Armonk, NY, USA). Continuous variables are expressed as the mean ± standard deviation or median (interquartile range), as appropriate. Categorical variables are summarized as counts and percentages in each category. Descriptive statistics were used to characterize the cohort.

Results

1. Baseline characteristics

From January to October 2020, 328 cancer patients with COVID-19-like symptoms were admitted to five hospitals in Daegu. Table 1 shows the baseline characteristics of the patients. The median age was 67 years (range, 14–99 years) with 126 patients (38.4%) aged > 70 years. A total of 191 patients (58.2%) were males. The four most common COVID-19-like symptoms were fever (n = 256, 78.0%), cough (n = 52, 15.9%), diarrhea (n = 19, 5.8%), and sore throat (n = 6, 1.8%). Comorbidities included hypertension (n = 134, 40.9%), diabetes mellitus (n = 91, 27.7%), and heart disease (n = 20, 6.1%).

2. Tumor characteristics

The most common solid malignancies were lung (n = 65, 19.8%) and hepatobiliary (n = 61, 18.6%) cancers (Table 2). Sixty-two patients (18.9%) had hematologic malignancies. Most patients (91.5%) received at least one antitumor therapy; 244 (74.4%) received cytotoxic chemotherapy, and 111 (33.8%) underwent surgical resection for tumor control. Based on the last response evaluation of the patients, 22 patients (6.7%) showed complete response, 39 (11.9%) showed partial response, 58 (17.7%) had stable disease, and 92 (28.0%) experienced progressive disease.
Among the patients tested for COVID-19, only three (1.2%) were confirmed to have COVID-19 based on a positive severe acute respiratory syndrome coronavirus (SARS-CoV)-2 PCR test result (Table 3). Most patients (253, 98.8%) with fever were not diagnosed with COVID-19. Of the sputum, blood, urine, and body fluid samples, 3.9%, 1.2%, 0.4%, and 0.8%, respectively, were culture-positive. More than 90% of the patients were neither diagnosed with COVID-19 nor culture-positive. Thirty-three patients (12.9%) diagnosed with neutropenic fever.

Twenty patients with fever experienced a delay in receiving cancer treatment, with a median delay of 17.5 days (range, 3–33 days). Eighteen patients discontinued active cancer treatment because of fever. Major events during the treatment delay period included death (2.7%), cancer progression (1.5%), and major organ dysfunction (2.7%). Two patients died of COVID-19. Twenty-eight patients (8.5%) died of cancer progression.

4. Characteristics of cancer patients diagnosed with COVID-19

Table 4 shows the characteristics of the patients with cancer diag-
Table 3. Cause of fever and treatment delay affected by fever

<table>
<thead>
<tr>
<th>Factor</th>
<th>Data (n = 328)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of fever (n = 256)</td>
<td></td>
</tr>
<tr>
<td>COVID-19</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Other infection</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>10 (3.9)</td>
</tr>
<tr>
<td>Bloodstream infection</td>
<td>3 (1.2)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1 (0.4)</td>
</tr>
<tr>
<td>Body fluid infection</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Neutropenic fever</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Neutropenic fever</td>
<td>33 (12.9)</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
</tr>
<tr>
<td>COVID-19</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Cancer progression</td>
<td>5 (1.5)</td>
</tr>
<tr>
<td>Delay of admission</td>
<td>17 (5.2)</td>
</tr>
<tr>
<td>Major organ dysfunction</td>
<td>9 (2.7)</td>
</tr>
<tr>
<td>Cause of death</td>
<td></td>
</tr>
<tr>
<td>COVID-19</td>
<td>2 (0.6)</td>
</tr>
<tr>
<td>Cancer progression</td>
<td>28 (8.5)</td>
</tr>
<tr>
<td>Sepsis</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>5 (1.5)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>3 (0.9)</td>
</tr>
</tbody>
</table>

Values are presented as number (%).

nosed with COVID-19. Of the three patients, two died. Two patients were identified as members of a religious group called ‘Shincheonji.’ A 71-year-old man with hypertension, chronic obstructive pulmonary disease, and heart disease was diagnosed with stage II lung cancer in January 2020. He presented with COVID-19 pneumonia after undergoing cytotoxic chemotherapy and died of COVID-19. An 82-year-old man with a history of laryngeal cancer was diagnosed with COVID-19 and recovered. A 73-year-old woman was simultaneously diagnosed with COVID-19 and lymphoma and died of COVID-19.

Discussion

Cancer patients are a vulnerable population during the ongoing COVID-19 pandemic [15-17]. They are at a high risk of SARS-CoV-2 infection and are more likely than other COVID-19 patients to develop severe disease [2]. In this study, we analyzed 328 cancer patients admitted to five hospitals in Daegu to investigate the incidence of COVID-19 in cancer patients with COVID-19-like symptoms.
Malignancy is well known to cause fever and neoplastic fever, which are frequently encountered in febrile cancer patients without other causes of fever and presents a diagnostic challenge in differentiating whether fever is attributable to infection, therapy, or disease [10]. In our study, the most common COVID-19-like symptom was fever. However, among the 328 patients with COVID-related symptoms, only three patients were diagnosed with COVID-19 (0.9%). The cause of fever in patients without COVID-19 or positive culture results was considered to be neoplastic fever. It may be challenging to differentiate COVID-19 from other infections and neoplastic fever. However, some factors that may be helpful in discerning between COVID-19 infection and other causes of fever are as follows: (1) COVID-19-related fever usually occurs between 2 and 14 days following exposure. (2) Neoplastic fever frequently occurs in patients with specific cancer subtypes such as lymphoma, leukemia, renal cell carcinoma, and sarcoma. (3) The most common symptoms that occur with neoplastic fever are diaphoresis, but less often include chills and rigor. In contrast, infectious fevers tend to present with warmth, diaphoresis, and chills, reflective of peripheral vasodilation. (4) Hypotension and tachycardia are commonly accompanied by systemic infections caused by gram-negative organisms [18].

Approximately 15% of cancer patients with fever experienced a delay in receiving chemotherapy. Eight patients died during the treatment delay period, six patients showed cancer progression, and eight patients experienced major organ dysfunction. A total of 33 patients died during the study period. Two of them died of COVID-19, and the other died of cancer-related complications. Cancer patients receiving adjuvant or palliative chemotherapy were advised to continue their treatment following the guidelines for cancer care during the COVID-19 pandemic, and clinicians were advised to implement treatment strategies with fewer hospital visits. However, patients had a tendency to frequently visit the hospital not only because of their symptoms but also due to anxiety. Patients' anxiety and physicians' difficulty in determining the cause of fever led to excessive testing for COVID-19 [19].

Considering that only 0.9% of patients tested for COVID-19 were positive, and the postponement of the chemotherapy schedule could lead to significant adverse events such as death or cancer progression, this study shows that an adequate screening test for COVID-19 in cancer patients with fever based on physicians' clinical decisions can improve their outcome.

The current study had certain limitations, including its retrospective nature and small sample size. However, the present results suggest that during the COVID-19 pandemic period, the approach to cancer patients with fever should be different from that before the pandemic period. Excessive testing for COVID-19 is not the best way to improve the prognosis of patients. Adequate testing for COVID-19 and adherence to COVID-19 prevention guidelines, including handwashing and wearing masks, are crucial for improving the prognosis of cancer patients during the COVID-19 pandemic.

Notes

Ethical statements
All procedures were performed in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards. The study was approved by the Institutional Review Board of five hospitals (Kyungpook National University Chilgok Hospital, Yeungnam University Medical Center, Daegu Fatima Hospital, Daegu Catholic University Medical Center, and Keimyung University Dongsan Medical Center) in Daegu (No. KNUH 2020-03-044, CR21-024, 2020-09-100, 2020-10-006), which waived the requirement for informed consent.

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

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Author contributions
Conceptualization: IHL, JYK; Data curation: IHL, SAK, SJL, SAL, YYC, JYL; Formal analysis: IHL, SAK, SJL, YYC, JYL, JYK; Supervision, Funding acquisition: JYK; Writing-original draft: IHL; Writing-review & editing: SAL.

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References


Anti-N-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma in Korea: three case reports

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Introduction

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a severe autoimmune paraneoplastic syndrome associated with ovarian teratomas. Most patients develop neurologic symptoms, including psychosis, memory deficits, seizures, or abnormal movements, and experience abdominal pain related to ovarian neoplasm. We present a case report of three patients diagnosed with anti-NMDAR encephalitis accompanied by ovarian teratomas at Ajou University Hospital in Korea. The patients demonstrated a different clinical course of the disease. However, upon diagnosis, all patients underwent surgical removal of the ovarian teratoma followed by intensive immunotherapy. The symptoms progressively improved following treatment. This is a case report of a rare autoimmune anti-NMDAR encephalitis associated with ovarian neoplasms, including immature teratoma.

Keywords: Anti-N-methyl-D-aspartate receptor encephalitis; Immature ovarian teratoma; Mature ovarian teratoma

Case report

Anti-N-methyl-D-aspartate receptor (NMDAR) encephalitis is a severe autoimmune paraneoplastic syndrome associated with ovarian teratomas. Most patients develop neurologic symptoms, including psychosis, memory deficits, seizures, or abnormal movements, and experience abdominal pain related to ovarian neoplasm. We present a case report of three patients diagnosed with anti-NMDAR encephalitis accompanied by ovarian teratomas at Ajou University Hospital in Korea. The patients demonstrated a different clinical course of the disease. However, upon diagnosis, all patients underwent surgical removal of the ovarian teratoma followed by intensive immunotherapy. The symptoms progressively improved following treatment. This is a case report of a rare autoimmune anti-NMDAR encephalitis associated with ovarian neoplasms, including immature teratoma.

Cases

1. Case 1
An 18-year-old woman presented with abdominal pain associated with a 6 cm left ovarian teratoma observed by an abdominal computed tomography (CT) scan (Fig. 1A). Surgical removal was planned. However, on the day of admission, she experienced se-
vere insomnia, followed by global amnesia, aggressive behavior, and suicidal ideation. The patient had headaches and memory disturbance for 2 weeks before admission (Table 1). As a result, the planned operation was postponed. Electroencephalogram (EEG) and brain magnetic resonance imaging (MRI) were unremarkable. Intensive antipsychotic treatment did not improve the symptoms. Under the suspicion of anti-NMDAR encephalitis, removal of the ovarian teratoma was performed (Fig. 1B). Both serum and CSF samples were found to be positive for antibodies against NMDAR. The histopathological diagnosis of the ovarian tumor was an immature teratoma (Fig. 1C, 1D). Immunoglobulin treatment and four cycles of rituximab were administered. The symptoms progressively improved, and at the 9-month follow-up, whole-body positron emission tomography CT and transvaginal sonography were normal.

2. Case 2
A 41-year-old woman was admitted to the hospital with fever and headache. The serum and CSF examinations showed no signs of inflammation. The patient complained about episodes of self-talking and disorientation for the last few days before admission. Brain MRI and EEG were normal. Despite intensive treatment, the clinical symptoms did not improve. Under the impression of anti-NMDAR encephalitis, an abdominal CT scan was performed (Fig. 2A). It showed a 4 cm ovarian teratoma that was immediately removed, and the patient received corticosteroids and immunoglobulin treatment (Fig. 2B). Anti-NMDAR antibodies were detected in serum and CSF samples. The pathologic diagnosis of the ovarian tumor was a mature teratoma (Fig. 2C, 2D). There was no evidence of recurrence at the 2-month follow-up.

3. Case 3
A 29-year-old woman experienced confusion, agitation, and auditory hallucinations. The serum blood test, CSF examination, and brain MRI showed no abnormalities. Only the EEG test revealed cerebral dysfunction. Empirical treatment with high-dose immunoglobulin, methylprednisolone, and plasma exchange was started; however, symptoms did not improve. Anti-NMDAR encephalitis was considered, and a 3 cm left ovarian teratoma was detected on an abdominal CT scan (Fig. 3A). The serum and CSF tested positive for anti-NMDAR antibodies. The tumor was removed surgically, and the patient received corticosteroids, plasmapheresis,
Fig. 2. Imaging and pathology findings for case 2. (A) Abdominal computed tomography findings. A 4-cm cystic mass with fat component (arrow) suggesting a teratodermoid tumor of the left ovary. (B) Intraoperative image of a left ovarian cyst. (C) Mature cystic teratoma shows a small amount of mature neuroglial tissue (arrows) with mature skin adnexal tissue, fat, and choroid plexus (hematoxylin and eosin stain, ×100). (D) Glial fibrillary acidic protein immunostaining highlights mature neuroglial tissue (arrows) in the mature cystic teratoma (immunohistochemical stain, ×100).

Table 1. Clinical characteristics of patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex/age (yr)</td>
<td>Female/18</td>
<td>Female/41</td>
<td>Female/29</td>
</tr>
<tr>
<td>Histology of ovarian tumor</td>
<td>Immature teratoma, grade III</td>
<td>Mature teratoma</td>
<td>Mature teratoma</td>
</tr>
<tr>
<td>Site/size of ovarian tumor (cm)</td>
<td>Left/6.4</td>
<td>Left/4.0</td>
<td>Left/3.0</td>
</tr>
<tr>
<td>Predromal symptom</td>
<td>Headache, memory disturbance</td>
<td>Headache, fever</td>
<td>Headache, fever, myalgia</td>
</tr>
<tr>
<td>Clinical symptom</td>
<td>Night sweats, global amnesia, aggressive behavior, depression, suicidal ideation</td>
<td>Self-talking, disorientation</td>
<td>Emotional instability, disorientation, dyskinesia, confusion, agitation, auditory hallucinations</td>
</tr>
<tr>
<td>Brain MRI</td>
<td>Normal</td>
<td>Increased T2 signal</td>
<td>Normal</td>
</tr>
<tr>
<td>CSF examination</td>
<td>Increased glucose</td>
<td>Increased protein</td>
<td>Increased glucose</td>
</tr>
<tr>
<td>EEG examination</td>
<td>Normal</td>
<td>Normal</td>
<td>Diffuse cerebral dysfunction</td>
</tr>
<tr>
<td>Medical treatment</td>
<td>Corticosteroids, IVIG, rituximab</td>
<td>Corticosteroids, IVIG, aciclovir</td>
<td>Corticosteroids, IVIG, rituximab, plasma exchange</td>
</tr>
<tr>
<td>Interval (day)</td>
<td>Between surgery and the onset of initial symptoms</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Between surgery and clinical improvement</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Follow-up (mon)</td>
<td>8</td>
<td>2</td>
</tr>
</tbody>
</table>

MRI, magnetic resonance imaging; CSF, cerebrospinal fluid; EEG, electroencephalogram; IVIG, intravenous immunoglobulin.
and rituximab thereafter (Fig 3B). The pathologic diagnosis of the ovarian tumor was a mature teratoma (Fig. 3C, 3D). She completely recovered within 3 months.

Discussion

Anti-NMDAR encephalitis is an autoimmune encephalitis first reported by Dalmau et al. [4,5] in 2007. It typically begins with prodromal symptoms such as nonspecific fever, diarrhea, vomiting, headache, or upper-respiratory symptoms before the onset of psychiatric and neurological symptoms, which are often accompanied by persistent amnesia. It is known that age, sex, and ethnicity are important factors related to the disease, as most patients are women, especially of Asian ethnicity [6,7]. NMDAR hypofunction is related to cognitive defects, whereas overstimulation causes excitotoxicity and subsequent neurodegeneration [8].

The clinical features that distinguish anti-NMDAR encephalitis from other neurological or psychiatric disorders are acute onset of mood and behavioral changes with no prior history of seizures or abnormal movements. The differential diagnoses include primary psychiatric disorder, drug abuse, neuroleptic malignant syndrome, migraine, antipsychotic medications, or infectious encephalitis.
Anti-NMDAR encephalitis should be considered once the above conditions are excluded [9]. The detection of immunoglobulin G antibodies in the serum or CSF confirms the diagnosis. In the advanced stages, the CSF antibodies usually remain elevated, while serum antibodies substantially decline with treatment [10]. There is limited evidence regarding the relationship between the size of the adnexal tumor and encephalitis. However, ovarian teratomas in anti-NMDAR encephalitis are usually not large, and an ovarian teratoma as small as 6 mm in diameter has been reported in association with anti-NMDAR encephalitis [11].

Treatment is focused on resection of the tumor followed by immunotherapy, including glucocorticoids, immunoglobulin, and plasma exchange, which collectively improves the symptoms within 4 weeks. For patients who do not respond to first-line immunotherapy, rituximab or cyclophosphamide may be used. Immunotherapy is applied to treat encephalitis regardless of the type of ovarian teratoma.

This is a case report of a rare autoimmune anti-NMDAR encephalitis associated with ovarian tumors, including immature teratoma, in Korea. Patients showed a wide spectrum of initial symptoms, and immediate surgical removal of the adnexal tumor was performed in all patients, followed by intensive immunotherapy. The course of the disease correlated with previous findings in that neurological symptoms progressively improved following the operation. Consciousness improved first, and then, autonomic function recovered. The frequency of epileptic seizures was reduced, and the social ability returned to the state before the onset of the disease.

Although we were not able to compare the clinical symptoms among anti-NMDAR encephalitis patients with or without ovarian teratoma, according to the study by Zhang et al. [12], there may be differences in laboratory and clinical findings between anti-NMDAR encephalitis with or without ovarian teratoma [12]. Anti-NMDAR encephalitis patients without ovarian teratoma experience more viral prodromal symptoms, whereas patients with teratoma present with milder neurological symptoms. In our study, two patients presented with fever and myalgia, mimicking viral infection, whereas one patient complained about memory disturbance. Moreover, the prevalence of immature teratomas, which generally comprise less than 1% of ovarian teratomas, is much higher among patients with anti-NMDAR encephalitis compared to the general population [13]. Therefore, it is important to inform patients about the possibility of malignancy in cases of anti-NMDAR encephalitis with ovarian teratoma.

In conclusion, anti-NMDAR encephalitis is a rare yet serious disorder that must be considered in patients with ovarian teratoma and neurological symptoms. Anti-NMDAR encephalitis may be speculated in patients who present with psychotic symptoms that are not improved by typical antipsychotic treatments, and the presence of an ovarian tumor detected on imaging studies. A comprehensive evaluation, including blood tests, CSF examination, brain and abdomen imaging, and antibody screening, is required to exclude other causes before making the diagnosis.

Once the disease is suspected, a combination of surgical removal of adnexal tumors and immunotherapy should be applied. The disorder can be fatal without appropriate treatment. Therefore, it should be included in the differential diagnosis in patients with ovarian teratoma and neurological symptoms.

Notes

Ethical statements
This retrospective study was approved by the Institutional Review Board (IRB) of the Ajou University Hospital (IRB No: MEDEXP-20-531), and the requirement for informed consent from the patient was waived by the IRB.

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

Author contributions
Conceptualization, Resources: JL, SK, HJC, YHL, JHS, TWK, SJC, KJH, MK; Data curation: TWK; Investigation: JL; Validation: KJH; Formal analysis, Supervision: SJC, KJH; Writing-original draft: JL; Writing-review & editing: MK.

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Cardiopulmonary bypass preparation is mandatory in cardiac exploration for blunt cardiac injury patients: two case reports

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Introduction

Blunt cardiac injury (BCI) following chest trauma is one of the most devastating injuries related to vehicular accidents that cardiac surgeons encounter. About 70% of blunt cardiac injuries occur in the right atrium (RA) and the right ventricle (RV) [1,2]. Although certain injuries may be repairable without cardiopulmonary bypass (CPB), it is difficult to identify the bleeding point preoperatively.

Here, we report two cases; pulmonary vein (PV) injury and left atrial appendage rupture as a result of blunt chest trauma. This report conveys the patient’s survival is depending on the maintenance of hemodynamic stability through CPB during the operation.

Cases

1. Case 1
A 70-year-old man with hypertension was transferred to our center after a traffic accident. He was in drowsy mental state with a Glasgow Coma Scale of 14. The patient was showing symptoms of chest pain and shortness of breath. Respiratory rate of 21 breaths per minute with an oxygen saturation of 97%, a regular pulse of 101 beats per minute, and blood pressure of 101/61 mmHg was recorded at the moment.

Computed tomography (CT) after the accident showed a small amount of hemopericardium, but no other vital organ injuries (Fig. 1). Based on these findings, BCI was strongly suspected. Transthoracic echocardiography (TTE) was performed by an experienced cardiologist, which revealed a small pericardial effusion,
but the site of injury could not be determined.

Under general anesthesia, exploratory median sternotomy was performed, and initial vital signs in the operating room were stable. The hematoma in the pericardium and over the RV was removed. In an attempt to visualize the LA and the apex of the left ventricle (LV), sudden massive arterial bleeding occurred. Subsequently, cardiac arrest ensued. While manual compression to the heart was being performed, CPB was established immediately with ascending aortic perfusion and two-staged right atrial venous drainage. CPB circuit priming and cannulation took approximately 20 minutes and open cardiac massage was continued. After achieving cardioplegic arrest, optimal visualization of the operative field was achieved.

Careful inspection of the heart revealed a 2-cm laceration of the left upper PV at its junction with the left atrium and the tear was repaired using a Peri-Guard Repair-Patch (Lamed GmbH, Berlin, Germany) and reinforced with 6-0 prolene (Fig. 2). Thorough inspection was performed again under CPB and no further injury was found. However, CPB weaning was difficult due to akinesia of the left anterior descending artery territory. We performed emergency coronary artery bypass surgery and subsequently weaned the patient off CPB. However, he remained in a semi-coma state after cardiac exploration and suffered low cardiac output syndrome (LCOS). Patient’s condition worsened and the patient was pronounced dead on postoperative day (POD) 2. Total aortic cross-clamp and CPB times were 112 and 208 minutes, respectively.

2. Case 2

A 61-year-old man was struck by a motor vehicle while riding his motorcycle and was admitted to our center. The patient’s initial blood pressure and heart rate were 124/72 mmHg and 85 beats per minute, respectively with alert mental status. The CT in the emergency room revealed scanty hemopericardium and right pleural effusion (Fig. 3). Associated injuries were descending colon mesenteric laceration and left distal radius fractures. There were no associated intracranial injuries. RV injury was suspected based on a significant amount of hematoma between the RV and the sternum on CT and decided to perform emergency exploration. However, to avoid unexpected catastrophic situations that may involve hemodynamic instability, CPB was prepared in advance. Through a median sternotomy approach, all the visible blood was removed; however, the active bleeding point was unable to be found. Remaining blood at the base of the heart was visible. In order to thoroughly examine this area, the decision was made to undergo the exploration under CPB assistance. CPB was established by cannulating the ascending aorta and the RA. Venting was performed by
placing a cannula into the LA and connecting this to the venous drainage line. After being guaranteed hemodynamic stability, a careful heart inspection was performed by elevating the LV apex. There was bleeding on the LA side coming from a 1.5-cm LA appendage rupture. We repaired the appendage laceration using 4-0 polypropylene suture with polytetrafluoroethylene felt pledget reinforcement, carefully avoiding the left circumflex artery (Fig. 4).

No intraoperative event occurred on weaning off bypass. After routine closure, exploratory laparotomy was performed. Aortic cross-clamp and CPB times were 38 and 60 minutes, respectively. The postoperative course was uneventful, and the patient was discharged on the POD 16 after radius fixation operation.

Discussion

BCI due to chest trauma is a fatal condition. Blunt chest trauma occurs up to 50% of all fatal motor accidents and it is known as the primary cause of death in 12% to 25% of such accidents [3]. In a recent report, BCI was seen in 0.045% of blunt trauma patients, with a mortality rate of 89% [1]. One autopsy study of 1,597 fatalities from blunt trauma identified cardiac injuries in 190 individuals (11.9%) [4]. Most BCIs occur due to motor vehicle crashes (approximately 50%), followed by pedestrians being struck by vehicles (35%) and motorcycle crashes (9%), while the remainders are mostly secondary to falls from a significant height [4,5].

Owing to its anterior location, the RV is the most commonly injured chamber in 40% of BCI patients. The RA and LV follow closely with 30% to 33% of all injuries, PV and LA tears occur in about 1% [2,5]. The mechanism of injury to the venous-atrial confluence, such as the PV is believed to be due to rapid deceleration that allows relatively mobile ventricles to move forward or laterally while the tethered posterior veins do stay stationary [2,4,6,7].
ferred to the advantage of using CPB in these cases [6,8-10,12]. When number and location of bleeding points are uncertain, median sternotomy is beneficial as it permits an easy approach to both sides of the heart as well as easy access to CPB [13-15]. Full examination and repair of the heart and great vessels are easier with CPB because of the bloodless field, decompressed heart, and reduced risk of hemodynamic instability due to sudden blood loss [8]. However, the presence of intracranial bleeding, which is aggravated by complete heparinization, limits the use of CPB [6].

In the second case, hemoperitoneum was present, however, heparinization did not have a significant effect on bleeding. Therefore, we believe that a short duration of CPB does not increase the risk of bleeding without an existing intracranial bleed.

In the first case, there was not much hemopericardium and vital signs were stable after arrival, minor RV tearing behind the sternum was suspected. However, the moment the heart was lifted to examine the base, excessive bleeding and cardiac arrest occurred. Although the patient’s femoral vessels were prepared for CPB for such an unexpected situation, it took a considerable amount of time to prime the CPB circuit and to carry out the cannulation.

In the second case, CPB was performed under the suspicion that the heart base could bleed out and successfully identified cardiac structures without compromising hemodynamics. This report can be used to determine the patient’s prognosis which may be different depending on whether or not stable hemodynamics is obtained upon identifying cardiac structures.

In our trauma center, there have been several cases that underwent cardiac exploration for suspicious BCI after blunt chest trauma (Table 1). Between January 2010 and August 2019, 15 patients underwent cardiac exploration at Kyungpook National University Hospital. Most of these are RA and RV injuries, and CPB was used in seven cases, for which hemodynamic stability was obtained while the cardiac injury was repaired. In three cases, patients had a small hemopericardium and stable vital signs upon arrival, but catastrophic hemorrhage occurred during full cardiac examination, resulting in hurried CPB access. Among them, two patients died from LCOS. In our experience, about 50% of blunt cardiac injuries might be reparable without CPB, but it is difficult to identify the bleeding point preoperatively. Based on this report, the resultant excessive bleeding in the process of examining the full cardiac structure may be managed successfully in hemodynamically stable patients. Therefore, we determined that it is risky to perform cardiac exploration without CPB.

In conclusion, BCI cases require careful evaluation of the initial CT or TTE and a therapeutic plan that prevents a devastating injury that could result in death. To treat this potentially lethal injury successfully, it is important to maintain hemodynamic stability while inspecting injured cardiac structures, and CPB through a median sternotomy can be a crucial role in saving the patients’ lives. This case report may help with decision making when confronting BCI patients who are in need of cardiac exploration.

Table 1. Data of blunt cardiac injury patients with blunt chest trauma in KNUH

<table>
<thead>
<tr>
<th>Sex/age (yr)</th>
<th>Cause</th>
<th>Amount of hemopericardium</th>
<th>Approach</th>
<th>Injury site</th>
<th>Cardiac arrest in OR</th>
<th>Use of CPB</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/46</td>
<td>Car accident</td>
<td>Small</td>
<td>Median sternotomy conversion in thoracotomy</td>
<td>LA</td>
<td>Yes</td>
<td>Yes</td>
<td>Death</td>
</tr>
<tr>
<td>Male/55</td>
<td>Car accident</td>
<td>Small</td>
<td>Thoracotomy</td>
<td>RV</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Female/41</td>
<td>Car accident</td>
<td>Small–moderate</td>
<td>Median sternotomy</td>
<td>RA</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/26</td>
<td>Motorcycle accident</td>
<td>Small</td>
<td>Median sternotomy</td>
<td>Not found</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/56</td>
<td>Motorcycle accident</td>
<td>Small</td>
<td>Median sternotomy</td>
<td>RV</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/70</td>
<td>Car accident</td>
<td>Small</td>
<td>Median sternotomy</td>
<td>PV</td>
<td>Yes</td>
<td>Yes</td>
<td>Death</td>
</tr>
<tr>
<td>Male/73</td>
<td>Car accident</td>
<td>Moderate</td>
<td>Median sternotomy</td>
<td>IVC</td>
<td>Yes</td>
<td>Yes</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/61</td>
<td>Motorcycle accident</td>
<td>Small</td>
<td>Median sternotomy</td>
<td>LA appendage</td>
<td>No</td>
<td>Yes</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/50</td>
<td>Motorcycle accident</td>
<td>Large</td>
<td>Median sternotomy</td>
<td>RV</td>
<td>No</td>
<td>Yes</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/63</td>
<td>Car accident</td>
<td>Small</td>
<td>Thoracotomy</td>
<td>RA</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/57</td>
<td>Car accident</td>
<td>Small</td>
<td>Median sternotomy</td>
<td>RA, IVC</td>
<td>No</td>
<td>Yes</td>
<td>Discharge</td>
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<tr>
<td>Female/44</td>
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<td>Moderate</td>
<td>Median sternotomy</td>
<td>IVC</td>
<td>No</td>
<td>Yes</td>
<td>Discharge</td>
</tr>
<tr>
<td>Male/49</td>
<td>Fall down</td>
<td>Moderate</td>
<td>Thoracotomy</td>
<td>LV</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Female/83</td>
<td>Fall down</td>
<td>Large</td>
<td>Median sternotomy</td>
<td>RV</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
<tr>
<td>Female/64</td>
<td>Animal attack</td>
<td>Small</td>
<td>Thoracotomy</td>
<td>RV</td>
<td>No</td>
<td>No</td>
<td>Discharge</td>
</tr>
</tbody>
</table>

KNUH, Kyungpook National University Hospital; OR, operating room; CPB, cardiopulmonary bypass; LA, left atrium; RV, right ventricle; RA, right atrium; PV, pulmonary vein; IVC, inferior vena cava; LV, left ventricle.

a) Case 1. b) Case 2.
Notes

Ethical statements
This study was approved by the Institutional Review Board (IRB) of the Kyungpook National University Hospital (IRB No: KNUH 2020-11-025-001). Written informed consent was obtained from the patient for publication of this case report and accompanying images.

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

Author contributions
Conceptualization: SAS, THO; Methodology: JYC, GJK, YOL; Writing-original draft: SAS, HJ; Writing-review & editing: SAS, HJ, THO.

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References
Safety and effectiveness of early cardiac rehabilitation in a stroke patient with heart failure and atrial fibrillation: a case report

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Stroke patients have reduced aerobic capacity. Therefore, intensive structured exercise programs are needed. We report the case of a patient with stroke and cardiac disease who underwent early inpatient cardiac rehabilitation (CR). A 38-year-old male patient with atrial fibrillation, heart failure, and cerebral infarction underwent a symptom-limited exercise tolerance test (ETT) without any problems on day 45 after admission. He completed a 2-week inpatient program and an 8-week home-based CR program. Follow-up ETT showed increased exercise capacity. The present case might be the first to report a safely performed CR program in a patient with stroke and cardiac comorbidity in Korea. Systematic guidance is needed for post-stroke patients to receive safe and effective CR for the secondary prevention of stroke and cardiovascular risk.

Keywords: Cardiac rehabilitation; Exercise; Heart failure; Stroke

Introduction

Stroke patients are predisposed to a sedentary lifestyle that leads to cardiorespiratory deconditioning, muscle atrophy, and further weakness [1]. The mean maximal oxygen uptake (VO₂ max) at 1 month after stroke has been reported to be approximately 60% of the normative values for sedentary healthy individuals, which is comparable to the previously reported age-adjusted VO₂ max at 1 month after myocardial infarction [2]. Patients with stroke reportedly have a lower aerobic capacity than patients with primary cardiac disease [3]. Furthermore, cardiac problems are often observed in patients with stroke. Among stroke survivors, approximately 28.8% present with coronary artery disease, and 16.5% have heart failure [4]. Patients with concomitant stroke and cardiac disease have lower aerobic capacity than patients with stroke alone [3]. Therefore, intensive structured exercise programs including aerobic and resistance training are needed for this group of patients. However, traditional stroke rehabilitation programs cannot provide sufficient exercise [1], and additional cardiac rehabilitation (CR) is needed.

Despite the importance of CR, the proportion of stroke patients enrolled in CR was 4.8% in a previous study [3]. To our knowledge, CR is not routinely indicated for individuals with stroke in Korea.

We report the case of a patient with stroke and cardiac disease who underwent early inpatient CR followed by home-based CR.

Case

A 38-year-old male patient who was a smoker (5 pack-years) and
had a history of unknown arrhythmia without medical treatment was admitted to the emergency room due to right hemiplegia. During the initial examination, the patient was stuporous with a Glasgow Coma Scale score of 7. He was diagnosed with infarction of the left middle cerebral artery (MCA) territory (Fig. 1A) with occlusion of the M1 segment of the left MCA (Fig. 1B). He had atrial fibrillation, heart failure with a left ventricular ejection fraction (LVEF) of 27% (Table 1), pulmonary edema, pleural effusion, and mild cardiomegaly (Fig. 2). He was admitted to the intensive care unit of the Department of Cardiology. He received a tracheostomy, mechanical ventilation, and additional medical treatment. He showed improvement in the LVEF from 27% to 47% on echocardiography and normal sinus rhythm on electrocardiogram (ECG) after treatment.

On day 37 after admission, the patient was transferred to the Department of Rehabilitation Medicine, where he was provided with stroke rehabilitation and CR. The Mini-Mental State Evaluation score denoting the cognitive function was 30. In the manual muscle test, the right upper extremity was graded as good in the proximal portion and fair in the distal portion. The right lower extremity was graded as good. The functional ambulation category (FAC) score was 4 and the Berg balance scale (BBS) score was 53. The modified Barthel index (MBI) score was 77. Speech evaluation showed anomic aphasia with an aphasia quotient (AQ) of 79. On day 38 after admission, the nasogastric tube was removed, and oral feeding was started after the videofluoroscopic swallowing study. The tracheostomy tube was also removed on day 43 after confirming that the patient had sufficient strength for coughing and sputum expectoration. He received physical therapy, occupational therapy, and speech therapy for stroke rehabilitation.

The first symptom-limited exercise tolerance test (ETT) was conducted on day 45 after admission (Fig. 3A). After 14 minutes and 48 seconds, the ETT was terminated upon patient’s request due to leg discomfort. Using the Fitness Registry and the Importance of Exercise National Database (FRIEND) equation, the pre-
dicted VO2 max, which reflected age and weight, was 42.15 mL/kg/min [5]. The measured VO2 max was 21.7 mL/kg/min (51.5% of predicted value), and there was no abnormality in exercise ECG and hemodynamic response. The patient participated in a CR program for 2 weeks (1-hour sessions five times per week) (Fig. 3B). An ECG-monitored exercise training with 4.6 metabolic equivalents (METs) was started, and the intensity was gradually increased. After 2 weeks, a follow-up ETT was performed, and the test was stopped after 15 minutes and 53 seconds upon patient’s request. The VO2 max improved from 21.7 to 27.3 mL/kg/min (from 51.5% to 64.8% of the predicted value) (Table 2). The patient also received an educational program about risk factor management, including smoking cessation and nutrition. Upon discharge (day 60 after admission), the BBS score changed from 53 to 56 (smaller than the minimal clinically important difference [MCID] of 12.5) [6], MBI score from 77 to 98 (larger than MCID of 20.1) [7], and AQ from 79 to 88.2.

The patient underwent a home-based CR program three times a week after discharge from the hospital for 8 weeks. After 8 weeks, a follow-up ETT was performed (Table 2). The test was stopped after 18 minutes and 30 seconds upon patient’s request, and the VO2 max showed further improvement (31.3 mL/kg/min, 74.3% of predicted value). Based on the guidelines of the American Association of Cardiovascular and Pulmonary Rehabilitation (AACVPR), risk stratification of the patient was changed from moderate risk at the first test to low risk at the follow-up ETT [8].

**Discussion**

Herein, an inpatient CR program was initiated on day 45 after admission in a patient with atrial fibrillation, heart failure, and stroke. There were no adverse events such as worsening of heart failure, cardiac arrest, or death during the 2-week exercise training. Subsequently, the patient’s exercise capacity and LVEF on echocardiography improved. A home-based CR program resulted in a further increase in exercise capacity.

Fig. 2. Chest X-ray shows pulmonary edema, pleural effusion, and mild cardiomegaly (arrows).

Fig. 3. (A) The photograph shows symptom-limited exercise tolerance test. (B) The photograph shows electrocardiogram-monitored exercise training.
Some previous studies have shown the effectiveness of CR in patients with stroke. Tang et al. [3] showed that a 12-month program providing aerobic and resistance training through a combination of supervised exercise sessions (once per week) and home exercise sessions (four times per week) improved the anaerobic threshold and VO₂ max in patients with concomitant stroke and cardiac disease and patients with stroke alone. These improvements were similar to the improvements observed in nonstroke participants. However, the study did not present any information regarding the interval between stroke and the beginning of CR. In another study [9], subjects having a transient ischemic attack or mild, non-disabling stroke within 12 months (mean of 11.5 weeks) were recruited to the outpatient CR program. Some of them had ischemic heart disease, but their percentages were not indicated. The 6-month CR program included 2-hour group sessions of risk factors, service education, and exercise training. The exercise training was performed on-site (50 sessions twice a week) or at home (at least four times a week). Upon program completion, a significant change of 2.04 METs (31.4%) was observed. Billinger et al. [10] studied the effect of an 8-week moderate-high aerobic exercise intervention in 10 patients with a diagnosis of stroke within 6 months (mean of 68.6 days) without any cardiac disease. There was a significant improvement in VO₂ max after the intervention. The results of our study are consistent with those of previous studies. However, an important finding of our study was that CR could be started quite early (day 45 after admission), considering the poor health condition of the patient.

Stroke patients in Korea generally receive stroke rehabilitation in both secondary and tertiary hospitals. These traditional stroke rehabilitation programs also include aerobic training but do not provide sufficient and maximal exercise because they are not based on ETT. They rather focus on motor recovery and gait exercise. Since stroke and cardiac diseases share many similar risk factors [1], intensive aerobic training, resistance exercise, and risk modification education are essential in stroke patients. If patients with stroke are provided with ETT and CR, the aerobic capacity and risk stratification of each patient will be calculated, leading to maximal, structured, and safe exercise programs. However, barriers to enhanced enrollment of stroke patients in CR include neurological and functional deficits such as hemiparesis and sensory ataxia, lack of a CR program mandate to include stroke patients, and personal perceived impact of stroke-related disability on participation [3]. Moreover, there is no indication of CR for stroke patients in the clinical practice guidelines for CR in Korea [11].

The safety of CR after acute cardiac disease has been suggested [12], but the safety of early CR in stroke patients has not been well elucidated. The present case is the first to report a safely performed CR program in a stroke patient with atrial fibrillation and heart failure in Korea. If a stroke patient has minor functional deficits as described in this case report (FAC 4 and lower extremity power good grade), CR provided with a traditional stroke rehabilitation program will lead to additional benefits. Systematic guidance is needed for post-stroke patients to receive safe and effective CR for the secondary prevention of stroke and cardiovascular risk.

**Notes**

**Ethical statements**

This retrospective study was approved by the Institutional Review Board (IRB) of Ulsan University Hospital (IRB No: 2021-01-024), and the requirement for informed consent from the patient was waived by the IRB.
Conflicts of interest
No potential conflict of interest relevant to this article was reported.

Author contributions
Conceptualization: all authors; Data curation, Visualization: SCL; Formal analysis: SCL, EJK; Methodology: EJK, JYL, ALH; Project administration: JYL, ALH; Investigation: SCL, JYL, ALH; Resources: JYL, ALH; Supervision: EJK; Writing-original draft: SCL; Writing-review & editing: EJK.

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References
Pembrolizumab-related autoimmune hemolytic anemia in a patient with metastatic lung adenocarcinoma: a case report

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Immune checkpoint inhibitors (ICIs) have become the main drugs for programmed cell death receptor-1 or ligand-1 expressing non-small cell lung cancer (NSCLC) combined with conventional chemotherapy. ICIs are generally more tolerable than cytotoxic chemotherapies in terms of toxicity, and ICI-related adverse events are mild and manageable. However, these drugs may lead to unexpected severe adverse events such as immune-related hematologic toxicities, which could be life-threatening. Here, a rare case of a pembrolizumab-related adverse event in a patient with NSCLC who showed early-onset hemolytic anemia and recovered by high-dose steroid and a series of plasma exchanges is reported.

Keywords: Autoimmune hemolytic anemia; Immune checkpoint inhibitors; Non-small-cell lung carcinoma; Pembrolizumab

Introduction

Efforts to turn on the immune system against cancers have led to the development of immune checkpoint inhibitors (ICIs) targeting programmed cell death receptor-1 or ligand-1 (PD-1/PD-L1) [1]. Since the first checkpoint molecular inhibitor ipilimumab was approved by the U.S. Food and Drug Administration (FDA), anti-PD-1/PD-L1 therapies are widely used in various cancers to improve survival outcomes, particularly in metastatic non-small cell lung cancer (NSCLC) and melanoma [2].

Although tumor cells escape immune attack through various complementary mechanisms of immunosuppression, ICIs targeting immunosuppressive molecules, such as cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and PD-1/PD-L1, can reactivate cytotoxic T cells to kill malignant cells [3]. Meanwhile, ICIs break the balance of the immune system, which may lead to the development of immune-related adverse events (IRAEs) in some patients. Generally, IRAEs can show variable autoimmune manifestations involving the skin, gastrointestinal tract, endocrine system, lung, joints, and many other organs [4]. Rarely, hematologic manifestations, such as cytopenias, have also been reported in patients with solid tumors and lymphomas during ICI treatment [5]. Most IRAEs are manageable by suppressing lymphocyte activation with steroids. However, some severe cases require changes in the treatment strategy [6].

Currently, pembrolizumab has become the first-line standard regimen for metastatic NSCLC with positive PD-L1 expression and negative actionable mutations for targeted therapies [7]. However, clinicians are faced with unexpected IRAEs and need more experience to overcome IRAEs. This paper reports a rare case of autoimmune hemolytic anemia (AIHA) in a patient with NSCLC...
receiving a pembrolizumab-containing regimen for metastatic lung cancer.

Case

A 70-year-old male newly diagnosed with metastatic NSCLC visited our oncology department in September 2020. The biopsied lung tissue revealed a poorly differentiated adenocarcinoma. Immunohistochemistry analysis showed overexpression of PD-L1 (100% by 22C3 pharmDx assay) and no alterations for targeted therapy including epidermal growth factor receptor, anaplastic lymphoma kinase, or c-ros oncogene 1. The patient’s general condition was good with an Eastern Cooperative Oncology Group performance status of 1, and he had no history of other diseases. He received pembrolizumab plus pemetrexed and cisplatin as first-line therapy for metastatic NSCLC based on international guidelines. His baseline complete blood cell count analysis at the beginning of chemotherapy revealed a white blood cell count of 11,450/μL, hemoglobin (Hb) 13.4 g/dL, and platelet (PLT) count of 218,000/μL.

Two weeks later, he complained of worsening dyspnea and was admitted for evaluation. Results of blood test showed Hb of 5.8 g/dL, total bilirubin of 4.16 mg/dL (direct bilirubin, 0.59 mg/dL and indirect bilirubin, 3.57 mg/dL), depletion of haptoglobin (< 10 mg/dL; range, 30–200 mg/dL), and increased serum lactate dehydrogenase (LDH) (478 U/L; range, 140–271 U/L). Over 20% of spherocytes were identified in the peripheral blood smear, and the direct antiglobulin test (DAT) and cold agglutinin test were positive (Table 1). With an increased reticulocyte count, we considered his severe anemia as AIHA, a rare IRAE of ICIs. The patient was administered prednisolone (1 mg/kg). Despite using high-dose steroids over a week, his LDH continuously increased over 1,000 U/L and PLT count decreased to < 100,000/μL without any sign of improvement in hemolytic anemia. Therefore, he was started on prednisolone at 2 mg/kg and underwent plasma exchange five times every other day. Features of hemolytic anemia showed significant improvement after 2 weeks of increased steroid dose and plasma exchange; therefore, the steroid dose was tapered slowly up to 10 mg/day while maintaining the normalized hemoglobin level. The patient then received subsequent conventional chemotherapy with pemetrexed plus cisplatin, and there was no recurrence of hemolysis. The overall treatment and progress are summarized in Fig. 1.

Discussion

Previous studies have reported that the frequency of immune-mediated cytopenias is < 0.5% in patients treated with ICIs [8]. According to the details of 68 AIHA cases identified in the FDA database, AIHA was relatively infrequent with pembrolizumab and ipilimumab compared to atezolizumab and nivolumab (Table 2) [9]. PD-1 inhibitor-associated AIHA has been reported to usually occur after two to five cycles of treatment compared with 8 to 12 weeks with CTLA-4 inhibitor therapy [10]. In the described case, the patient showed features of hemolytic anemia with positive DAT and the presence of cold agglutinin after only one cycle of pembrolizumab, including combination chemotherapy. Generally, a positive DAT is an important AIHA feature, and > 50% of immune-mediated anemia show positive DAT with immunoglobulin (Ig) G or C3. Cold agglutinin disease (CAD) is another form of AIHA that accounts for 10% to 20% of AIHA. Hemolysis of CAD is primarily extravascular, which is mediated by IgM, cold agglutinin, and complements at low temperatures [11]. Although pembrolizumab-associated AIHA with cold agglutinin and DAT is rare, a similar case of metastatic lung cancer has been reported [12].

The general recommendations for AIHA management include steroids and consideration of plasma exchange, and the guidelines on the management of ICI-related AIHA are similar. The key mechanism of IRAEs is that ICI therapies disrupt immunological homeostasis and reduce T-cell tolerance [13]. Therefore, the European Society of Medical Oncology suggests that high-dose corticosteroids and/or other immunosuppressive drugs should be considered in cooperation with a hematologist [14]. The American Society of Clinical Oncology recommends discontinuation of ICIs and commencement of prednisone (1–2 mg/kg/day) with red blood cell transfusion targeting Hb of 7 to 8 g/dL [15]. Previous studies have reported that immune-related anemia may be steroid-resistant, and 1 mg/kg of prednisone may be insufficient to resolve hemolysis [5,8]. In particular, some cases with nivolumab showed a poor response to steroids, resulting in fatal outcomes. Conversely, some cases with pembrolizum-

Table 1. Results of laboratory investigations corresponding to hemolytic anemia

<table>
<thead>
<tr>
<th>Investigation</th>
<th>Result (normal range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>5.8 (11–17)</td>
</tr>
<tr>
<td>Reticulocytes (×10^9/L)</td>
<td>160 (22–139)</td>
</tr>
<tr>
<td>Total bilirubin (mg/dL)</td>
<td>4.16 (0.3–1.2)</td>
</tr>
<tr>
<td>Lactate dehydrogenase (U/L)</td>
<td>478 (140–271)</td>
</tr>
<tr>
<td>Haptoglobin (mg/dL)</td>
<td>&lt; 10 (30–200)</td>
</tr>
<tr>
<td>Direct antiglobulin test</td>
<td>Positive</td>
</tr>
<tr>
<td>Cold agglutinin</td>
<td>Positive</td>
</tr>
</tbody>
</table>


https://doi.org/10.12701/yujm.2021.00899
Fig. 1. Time course of hemoglobin, bilirubin, serum lactate dehydrogenase (LDH), reticulocyte, and treatment.

Table 2. Proportion of AIHA in the OAEs and characteristics of patients who developed AIHA according to the FDA database

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ipilimumab</th>
<th>Nivolumab</th>
<th>Pembrolizumab</th>
<th>Atezolizumab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case of AIHA/OAE^a</td>
<td>7/12,631 (0.055)</td>
<td>43/20,335 (0.211)</td>
<td>13/8,917 (0.146)</td>
<td>5/2,021 (0.247)</td>
</tr>
<tr>
<td>Age (yr)^b</td>
<td>65 (32–68)</td>
<td>68 (43–85)</td>
<td>62 (35–82)</td>
<td>67 (57–69)</td>
</tr>
<tr>
<td>Underlying malignancy</td>
<td>MM: 7</td>
<td>LC: 18, MM: 17^c, HL: 3^c, RCC: 2, Others: 4</td>
<td>MM: 7, LC: 5, Others: 1</td>
<td>BC: 2, LC: 1, MM: 1, OC: 1</td>
</tr>
<tr>
<td>Other reported hematological adverse event with AIHA</td>
<td>Agranulocytosis, bicytopenia</td>
<td>ITP</td>
<td>ITP, PRCA</td>
<td>-</td>
</tr>
</tbody>
</table>

AIHA, autoimmune hemolytic anemia; OAE, overall adverse events; FDA, U.S. Food and Drug Administration; MM, malignant melanoma; LC, lung cancer; HL, Hodgkin lymphoma; RCC, renal cell carcinoma; BC, breast cancer; OC, ovarian cancer; ITP, immune thrombocytopenic purpura; PRCA, pure red cell aplasia.

^aNumber (%). ^bMedian (range). ^cOne patient had both HL and MM.
ab-related AIHA were relatively not severe and responded to steroids \[9,16,17\]. In our experience, there was little effect with 1 mg/kg of prednisone, and the features of severe hemolytic anemia began to improve by increasing the prednisone dose to 2 mg/kg/day. Maintaining high-dose steroids over a week with plasma exchange was also important in controlling hemolysis and preventing recurrence.

ICI therapy-associated AIHA is rare, but most cases can be life-threatening. Recently, the number of reported cases has increased with the expansion of ICIs and the number of patients exposed to them. Early recognition is important, and we hope that patients with ICI-related AIHA can receive appropriate management, including high-dose steroid and plasma exchange, through this case report. Further studies with immune cell profiling in patients with hematological IRAEs should be performed to better understand its mechanism and adequate management. In addition, trials including other ICIs with reportedly less hematological IRAEs for patients who experienced immune-related hemolytic anemia should be considered.

**Notes**

**Ethical statements**

This study was approved by the Institutional Review Board (IRB) of Kyungpook National University Chilgok Hospital (IRB No: KNUCH 2021-02-007). The patient provided written informed consent for publication of clinical details.

**Conflicts of interest**

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

**Author contributions**

Conceptualization, Data curation, Formal analysis, Methodology, Project administration, Visualization, Investigation, Validation: all authors; Resources, Supervision: YSC, Writing-original draft, Writing-review & editing: all authors.

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Introduction

Chronic pain reduces the quality of life, causes discomfort in daily living, and leads to a loss of labor [1]. Various mechanisms are involved in the development and amplification of chronic pain; consequently, multiple drugs are used in combination to control it effectively [2]. However, although it is generally accepted that analgesic drugs cause adverse effects on the liver, kidney, gastrointestinal, and cardiovascular systems, they can also result in serotonin syndrome (SS). It is a potentially life-threatening condition that is caused by the administration of drugs that increase serotonergic activity in the central nervous system [3,4]. Here, we aimed to show that clinicians should consider the potential for the development of SS in patients taking analgesic drugs, by presenting the case of a patient with chronic pain who developed SS, which was initially mistaken for a psychogenic nonepileptic seizure.

Case

A 36-year-old female visited the pain clinic in our university hospital for sudden onset agitation, abnormal movement, and hyperhidrosis (Supplementary Video 1). Three years previously, the patient had undergone surgical procedures (laminectomy and discectomy) owing to a herniated lumbar disc (HLD) on L4–5. After the

Serotonin syndrome (SS) is a potentially life-threatening condition that is caused by the administration of drugs that increase serotonergic activity in the central nervous system. We report a case of serotonin syndrome in a patient with chronic pain who was taking analgesic drugs. A 36-year-old female with chronic pain in the lower back and right buttock area had been taking tramadol hydrochloride 187.5 mg, acetaminophen 325 mg, pregabalin 150 mg, duloxetine 60 mg, and triazolam 0.25 mg daily for several months. After amitriptyline 10 mg was added to achieve better pain control, the patient developed SS, which was mistaken for psychogenic nonepileptic seizure. However, her symptoms completely disappeared after discontinuation of the drugs that were thought to trigger SS and subsequent hydration with normal saline. Various drugs that can increase serotonergic activity are being widely prescribed for patients with chronic pain. Clinicians should be aware of the potential for the occurrence of SS when prescribing pain medications to patients with chronic pain.

Keywords: Chronic pain; Depression; Prescription drug; Seizure; Serotonin syndrome
surgery, her pain due to HLD was almost relieved completely. However, 2 years after the surgery, she had a car accident; subsequently, she experienced lower back and right buttock pain (numeric rating scale, 8) that was not controlled despite receiving an epidural steroid injection in our pain clinic and taking various oral medications. She had been taking tramadol hydrochloride 187.5 mg, acetaminophen 325 mg, pregabalin 150 mg, duloxetine 60 mg, and triazolam 0.25 mg daily for several months. Five days prior to the visit to our pain clinic, to achieve better pain control, amitriptyline 10 mg was added at bedtime. Approximately 5 hours after taking amitriptyline 10 mg, the patient developed sudden agitation, abnormal movements, and hyperhidrosis.

One day before visiting our pain clinic, she visited the emergency room (ER) of our university hospital. The clinician in the ER diagnosed her with psychogenic nonepileptic seizure based on her history of visiting the psychology department for depression after the car accident and the agitation presented in the ER. Without performing further evaluation and treatment, the clinician discharged her to home. However, as the patient’s symptoms continued the next day, she visited our pain clinic. She presented with agitation and nervousness, tremor and myoclonus of bilateral lower limbs, rigidity, hyperhidrosis, dyspnea, and tachycardia (110 beats/min). Her body temperature (36.7°C) and blood pressure (120/70 mmHg) were within normal ranges. Based on Radomski criteria, SS was diagnosed [3], and the patient was admitted to our hospital. Laboratory tests performed to exclude other possible etiologies did not show any abnormalities: white blood cells (WBC), 6,480/μL; hemoglobin, 11.6 g/dL; C-reactive protein, 0.075 mg/dL; aspartate aminotransferase, 23 IU/L; alanine aminotransferase, 15 IU/L; creatinine, 0.51 mg/dL; urine WBC, 0–1/high-power-field; blood culture, negative; and chest X-ray, negative. She was hydrated with normal saline, and pregabalin, duloxetine, and triazolam were discontinued. Approximately 48 hours after admission, her symptoms had completely disappeared.

**Discussion**

We describe a case where the patient developed SS due to analgesic drugs. SS was diagnosed based on the Radomski criteria [3], which require the presence of at least four major symptoms or three major symptoms with two minor symptoms (major symptoms: confusion, elevated mood, coma or semi-coma, fever, hyperhidrosis, myoclonus, tremors, chills, rigidity, and hyperreflexia; minor symptoms: agitation, insomnia, tachycardia, tachypnea, diarrhea, low or high blood pressure, impaired coordination, mydriasis, and akathisia), and the historical coincidence of addition, or a recent increase in dosage of drugs that activate the serotonergic system. Of the symptoms included in the Radomski criteria, our patient’s symptoms fulfilled four major and two minor symptoms. In addition, the patient was administered several drugs that can activate the serotonergic system.

Nonepileptic seizures are characterized by sudden and time-limited disturbances of the motor, sensory, autonomic, emotional, and/or cognitive functions [5]. Symptoms of psychogenic nonepileptic seizures are similar to those of SS. However, unlike SS, the duration of nonepileptic seizures is usually brief, persisting 2 to 5 minutes [5].

Duloxetine and amitriptyline inhibit serotonin reuptake, which alleviates chronic pain through the attenuation of persistent pain mechanisms, such as central sensitization and hyperexcitability of the spinal and supraspinal pain-transmitting pathways [6]. The increased serotonergic activity caused by the action of duloxetine and amitriptyline can induce SS. In addition to the medications mentioned above, tramadol and opioids, popular choices for treating chronic pain, promote serotonin release and inhibit serotonin reuptake, and have been known to contribute to the development of SS [7]. Moreover, approximately 30% to 50% of patients with chronic pain have depression; many use antidepressants, which puts them at risk for the development of SS. As SS is a life-threatening condition, clinicians should be aware of the symptoms and, if patients taking pain medications exhibit these symptoms, drugs related to SS should be stopped immediately, and the patient should be hydrated. Clinicians also should be careful not to misdiagnose SS as psychogenic nonepileptic seizure.

**Supplementary materials**

Supplementary Video 1 can be found via https://doi.org/10.12701/yujm.2021.00948.

**Notes**

**Ethical statements**

This study was approved by the Institutional Review Board (IRB) of the Yeungnam University Hospital (IRB No: 2021-01-021). Written informed consent was obtained for publication of this case report and accompanying video.

**Conflicts of interest**

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

**Author contributions**

Conceptualization, Visualization, Methodology: All authors; Data

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curation, Formal analysis, Investigation, Supervision, Validation: MCC; Writing-original draft: All authors; Writing-review & editing: All authors.

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References

Anesthetic management during whole-lung lavage using lung ultrasound in a patient with pulmonary alveolar proteinosis: a case report

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²Department of Anesthesiology and Pain Medicine, Ulsan University Hospital, University of Ulsan College of Medicine, Ulsan, Korea

Pulmonary alveolar proteinosis (PAP) is an uncommon disease characterized by progressive accumulation of lipoprotein material in the lungs due to impaired surfactant clearance. Whole-lung lavage (WLL) is the current standard treatment and consists of sequential lavage of each lung to mechanically remove the residual material from the alveoli. Although WLL is considered safe, unexpected complications can occur. Moreover, due to the rarity of the disease itself, this procedure is unknown to many physicians, and management of intraoperative complications can be challenging for anesthesiologists. Lung ultrasound (LUS) provides reliable and valuable information for detecting perioperative pulmonary complications and, in particular, quantitation of lung water content. There have been reports on monitoring the different stages of controlled deaeration of the non-ventilated lung during WLL using LUS. However, it has been limited to non-ventilated lungs. Therefore, we report the use of LUS in WLL to proactively detect pulmonary edema in the ventilated lung and implement a safe and effective anesthesia strategy. Given the limited diagnostic tools available to anesthesiologists in the operating room, LUS is a reliable, fast, and non-invasive method for identifying perioperative pulmonary complications in patients with PAP undergoing WLL.

Keywords: Anesthesia; Extravascular lung water; One-lung ventilation; Pulmonary alveolar proteinosis; Ultrasonography

Introduction

Pulmonary alveolar proteinosis (PAP) is a rare disorder characterized by the accumulation of amorphous acellular lipoproteins in the alveoli, hindering gas exchange [1]. Whole-lung lavage (WLL) is the current standard treatment method consisting of sequential lavage of the affected lung through repeated filling and emptying cycles to remove excess alveolar phospholipids [2]. Although WLL is considered a safe and effective procedure for most patients with PAP, unexpected complications may arise. Knowledge of the physiological effects allows anesthesiologists to implement appropriate management [3].

Lung ultrasound (LUS) can help assess pulmonary and pleural pathologies in critically ill patients. It is now a standard technique for the early diagnosis of pulmonary edema in patients with heart failure, even in the subclinical stage [4]. There have been several reports on anesthesia management using LUS during WLL [5,6]. However, the use of LUS to monitor therapeutic procedures for both ventilated and non-ventilated lungs in the field of WLL is still in its infancy.
We hypothesized that the previously validated LUS images could help identify aeration changes in ventilated and non-ventilated lungs during WLL. This modality may allow for early complication recognition, such as spillover into the ventilated lung, pleural effusion, and pulmonary edema, in addition to guiding anesthetic management and the troubleshooting of intraoperative complications in patients with PAP undergoing WLL. Herein, we report a case with the aim to reduce complications such as alveolar hyperinflation and systemic absorption of saline and overdistension of alveoli by early detection of pulmonary edema in the ventilated lung using these characteristics of LUS.

**Case**

A 54-year-old male patient with a height and weight of 180 cm and 78 kg, respectively, presented to our hospital with a 2-month history of non-productive cough and progressive exertional dyspnea. The patient was a smoker (30 pack-years) without any other relevant medical history. Preoperative electrocardiography (ECG) and transthoracic echocardiography did not reveal any abnormalities. Chest radiography showed ill-defined bilateral opacities in the lower lung zones, and chest computed tomography showed extensive patchy ground-glass opacities in both lower lobes (Fig. 1). The left lung had more severe ill-defined opacities than the right lung. Preoperative pulmonary function testing results showed one-second forced expiratory volume (FEV₁) of 3.23 L (80% of predicted), forced vital capacity (FVC) of 4.11 L (78% of predicted), FEV₁/FVC ratio of 79%, total lung capacity (TLC) of 5.40 L (75% of predicted), and carbon monoxide diffusion capacity (DLco) of 49%. The patient exhibited a mild restrictive physiological pattern with decreased TLC and DLco. Cytological examination of the bronchoalveolar lavage fluid was suggestive of PAP, and pulmonologists planned for a WLL as first-line treatment.

Upon entering the operating room with a nasal cannula with 4 L/min O2, the patient was subjected to standard monitoring practices such as ECG, noninvasive blood pressure, bispectral index, and pulse oximetry. Before induction of anesthesia, all necessary equipment was checked and included 30 L of normal saline, a Y-piece connector, an irrigation set with clamps, a plastic container, and a rapid infusion system (RIS). Initial vital signs were stable at a blood pressure of 130/90 mmHg, heart rate of 90 beats/min, respiratory rate of 16 breaths/min, and temperature of 36.2°C. Initial arterial blood gas analysis (ABGA) showed a pH of 7.42, arterial partial pressure of carbon dioxide of 34 mmHg, arterial partial pressure of oxygen of 54 mmHg, bicarbonate concentration of 22.1 mmol/L, and arterial oxygen saturation of 88% on room air. The alveolar-arterial oxygen difference was 53 mmHg, suggesting gas exchange issues. After preoxygenation, the anesthesiologist intravenously administered 1% propofol (2 mg/kg) and rocuronium (0.6 mg/kg). Total intravenous anesthesia with propofol and

![Fig. 1.](https://doi.org/10.12701/yujm.2021.01284)
remifentanil was maintained throughout the procedure. A 37-French (Fr) left-sided double-lumen endotracheal tube (DLT) was used for one-lung ventilation (OLV). We confirmed proper tube placement by auscultation of lung sounds and fiber-optic bronchoscopy. Because the left lung was more affected, a right-sided OLV was performed. A manual leak test was performed by immersing the non-ventilated left lung tube in saline while ventilating the right lung. Radial arterial and jugular venous catheters were used to continuously assess arterial blood gas and hemodynamic status during the perioperative period. The mechanical ventilation parameter setting was as follows: tidal volume was 6 mL kg$^{-1}$ during two-lung ventilation (TLV), 4 mL/kg$^{-1}$ during OLV, with 5 cmH$_2$O positive end-expiratory pressure (PEEP) maintained throughout. Initially, WLL was started in the supine position. A Y-piece connected to the non-ventilated lung tube served as a link between the DLT and RIS. The warmed normal saline was then instilled into the left lung through the left tracheal lumen using the RIS at a rate of < 125 mL/min with a 30° head-up tilt (reverse Trendelenburg) position to facilitate the instillation (Fig. 2A). While draining the lavage fluid, the patient was placed in the head-down tilt (Trendelenburg) position (Fig. 2B), and the pulmonologist performed the process of chest percussion [1].

LUS was performed using a Versana Balance echograph and a 2–5 MHz convex array probe (GE Healthcare, Chicago, IL, USA). The patient was scanned in the supine position by a single anesthesiologist (JO). Three regions (phrenic point, upper and lower BLUE points) were identified bilaterally in each hemithorax according to the bedside LUS in emergency (BLUE) protocol (Fig. 3) [7]. Images were obtained at four intervals: (1) baseline (before anesthetic induction), (2) end lavage (after the introduction of the last bolus of normal saline into the non-ventilated lung), (3) revent-
tilation (after reinflation of the non-ventilated lung), and (4) pre-extubation (immediately before extubation). Six points were explored, and ABGA was performed in each of the four steps. Each finding was classified into three patterns according to previously reported LUS semiotics (Table 1) [2]. The lavage procedure was terminated when a tissue pattern (No. 3 in Table 1) appeared in all areas of the non-ventilated lung or three or more B-lines (No. 2 in Table 1) appeared in the ventilated lung without waiting for the fluid to overflow into the endotracheal tube. At the same time, serial ABGAs were performed to monitor hypoxemia, hypercapnia, acidosis, and electrolyte imbalance (Table 2). At the 9th cycle, an imbalance of more than 100 mL between infusion and drained volumes, as well as an increased number of B-lines of the ventilated lung, was observed compared to the previous cycle (II in Fig. 4). We managed with an adequate PEEP of 7 cmH₂O and negative water balance using intravenous diuretics (0.5 mg/kg intravenous

<table>
<thead>
<tr>
<th>No.</th>
<th>Pattern Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal/nearly normal pattern Pleural line, pleural sliding, A-lines (normal pattern), or B-lines in number &lt; 3 per lung ultrasound scan (nearly normal pattern)</td>
</tr>
<tr>
<td>2</td>
<td>Alveolar-interstitial syndrome B-lines in number ≥ 3 per lung ultrasound scan, more or less crowded, up to complete coalescence of these artifacts (the so-called &quot;white lung&quot;)</td>
</tr>
<tr>
<td>3</td>
<td>Alveolar consolidation Tissue-like pattern, with precise anatomical boundaries, no dimensional change throughout the respiratory cycle and variable depth extension</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variable</th>
<th>Basal</th>
<th>OLV 5</th>
<th>OLV 30</th>
<th>End lavage</th>
<th>Reventilation</th>
<th>Pre-extubation</th>
<th>Post-extubation</th>
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<td>PaO₂ (mmHg)</td>
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<td>76</td>
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<td>64</td>
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<td>PaCO₂ (mmHg)</td>
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<td>93</td>
<td>88</td>
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<td>98</td>
<td>96</td>
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<td>76</td>
<td>59</td>
<td>107</td>
<td>357</td>
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Table 1. Patterns describing lung ultrasound findings during whole-lung lavage

Table 2. Results of arterial blood gas analysis during left lung lavage

OLV, one-lung ventilation; OLV 5, within 5 minutes of OLV; OLV 30, within 30 minutes of OLV; PaO₂, arterial partial pressure of oxygen; PaCO₂, arterial partial pressure of carbon dioxide; HCO₃⁻, bicarbonate; SaO₂, arterial oxygen saturation; FiO₂, fraction of inspired oxygen; P/F ratio, PaO₂/FiO₂ ratio.

Fig. 4. The typical sequence of ultrasound findings of the lung. Our lavage procedure was terminated when a tissue pattern (pattern 3, II) appeared in all areas of the non-ventilated lung and/or three or more B-lines (pattern 2, II) appeared in the ventilated lung. Reventilation was associated with reduced B-lines in the ventilated lung and a reappearance of pattern 2 (alveolar-interstitial syndrome) in the lavaged lung. Prolonged ventilation with PEEP (18 hours later; ventilation with 10 cmH₂O PEEP) resulted in the return of pattern 1 (normal pattern) in both lungs. PEEP, positive end-expiratory pressure.
furosemide). The procedure was then terminated. Subsequently, TLV was initiated. Several B-lines were observed in the lavaged lung, whereas the ventilated lung showed a regular pattern with a marked decrease (III in Fig. 4). The total normal saline administered was 9.8 L, and 8.9 L saline was retrieved from the left lung. Intraoperative fluid management was performed with intermittent confirmation of the volume status by qualitative methods such as visual estimation using transthoracic echocardiography because of its technical complexity and time-consuming nature. The total volume of intravenously infused crystalloid solution administered to the patient during the WLL procedure was 1,850 mL, and the total urine output during the procedure was 450 mL. The DLT was exchanged for a 7.0 mm single lumen, and the patient was transferred to the intensive care unit. The next day, the patient was extubated. Manual chest physiotherapy was continued postoperatively. The symptoms improved clinically with a saturation of ≥ 94% on room air (Table 2), and he was discharged 1 week after the procedure.

Discussion

Our case report demonstrates the feasibility of using LUS during all phases of WLL. Although there are several reports of using LUS for monitoring procedures during WLL, little is known about the use of LUS in terminating the WLL process to prevent flooding of the ventilated lung. We identified an increase in the number of B-lines through LUS, suggesting pulmonary edema in the ventilated lung and efficiently determining the termination of WLL.

PAP is a diffuse lung disease characterized by the accumulation of amorphous, periodic acid-Schiff-positive lipoprotein material in the distal air spaces [8]. Treatment depends on the severity of the disease. The indications for WLL are decreased lung function, radiographic signs of disease progression, poor alveolar gas exchange, and worsening of respiratory symptoms. The contraindications are uncorrectable clotting disorders, high anesthetic risk, and cardio-pulmonary instability [3]. In our case, the patient underwent WLL because of the progressive worsening of respiratory symptoms. The left lung with a more severe disease was lavaged first in our patient, whereas the right lung was lavaged under general anesthesia after 1 month.

Although WLL is a widely used therapeutic procedure for patients with PAP, the short-term or long-term outcomes of the procedure are not well described. In general, approximately 10 to 15 lavages are performed to remove lavage effluent from each lung, each requiring 1 to 1.5 L of warm saline [9]. WLL is terminated when the lavage effluent appears clear on visual inspection of the protein content of the sample [3]. Specifically, if the patient’s hemodynamic status and oxygenation are tolerable, this process is repeated until the lavage effluent is washed out satisfactorily. Although the lavage method seems simple, the clinical results reported so far are diverse, ranging from complete resolution to death due to respiratory failure or pneumonia [10]. Some authors have mentioned delayed improvement or temporary worsening of lung function after WLL [11]. In addition, Huber et al. [12] described changes in respiratory mechanics and alveolar morphology after WLL in dogs. This procedure has also been shown to remove large amounts of surface-active substances [13] and other proteins [14]. In other words, saline lavage of the lungs until the lavage effluent is clear may not necessarily be a good thing. Moreover, the procedure of WLL is associated with complications such as pneumothorax, pleural effusion, hydropneumothorax, mediastinal shift, and increased intrathoracic and central venous pressures due to excessive lavage fluid, leading to hypotension. In particular, the risk of pulmonary edema in the ventilated lung is significantly higher in prolonged procedures such as WLL. Several mechanisms can cause pulmonary edema in the ventilated lung during the WLL procedure. Intraoperative fluid overload or lavage fluid leakage into the ventilated lung is a common cause. In addition, it is believed that changes in hydrostatic forces in the pulmonary microcirculation or activation of proinflammatory cytokines from the collapsed lung may cause pulmonary edema in the ventilated lung [15]. Therefore, since the non-ventilated lung is fully consolidated during WLL, mild pulmonary edema of the ventilated lung can significantly impact the prognosis of patients with preexisting PAP. This is an important reason for the early detection of pulmonary edema that occurs in ventilated lungs during the WLL procedure using LUS. Indeed, pulmonary edema by LUS has been evaluated in traumatic brain injury, acute respiratory distress syndrome, chronic renal failure, and cardiac surgery, with good sensitivity (97%) and specificity (98%) [4,16-18]. However, it has rarely been evaluated in the WLL procedure, especially in ventilated lungs, which may represent a potentially unique problem due to rapidly changing fluid dynamics. In our case, we confirmed an increase in the number of B-lines compared to baseline in ventilated lungs via LUS, which enabled early detection of pulmonary edema in ventilated lungs.

LUS has developed dramatically in the past decade, and its advantages include a lack of radiation and bedside accessibility. It has become a powerful tool for managing critically ill patients with acute respiratory failure, including diagnostic assessment, identifying the cause of acute gas exchange deterioration during ventilation support, and investigating weaning failure [19]. WLL represents a typical human model of lung aeration changes, similar to various pathological conditions in critically ill patients. The overall course of WLL, which includes OLV and progressive alveolar overflow, is quite similar to resorption atelectasis and lung consolidation. In
In this case report, LUS reliably monitored and recorded changes in lung aeration. Starting from a nearly regular pattern to apparent changes in lung deaeration throughout WLL were associated with significant changes in LUS findings in both ventilated and non-ventilated lungs. In the non-ventilated lung, the transition from normal to the alveolar-interstitial pattern was achieved during OLV, and continuous alveolar flooding promoted the transition to a consolidation pattern. Reventilation was more likely to result in a pattern of alveolar-interstitial syndrome due to residual water in the lavaged lung. In the ventilated lung, the number of B-lines progressively increased regularly as the unventilated one overflowed with saline. We considered this to be the endpoint of WLL. After adequate PEEP and negative water balance, the number of B-lines in the ventilated lung was significantly reduced, and it reappeared in a normal pattern. As in previous studies, terminating WLL when the lavage effluent is clear can be very subjective. In addition, because there are no randomized trials or formal prospective studies on the WLL procedure, it is difficult to determine the impact of current lavage methods on patient prognosis. In our case, as mentioned above, the endpoint of WLL was defined as the appearance of pulmonary edema in the ventilated lung. Our patient underwent similar anesthetic management using LUS for WLL in the contralateral lung after 1 month. Serial WLL resulted in clinical and physiological improvement in the patient. According to previous studies, and in 55% of patients with PAP, repeated lavage every 6 to 12 months may be required [20]. Moreover, in a significant number of patients, it is often impossible to implement continuous WLL due to hypoxemia or poor clinical conditions. Therefore, given the high burden of complications in patients undergoing WLL, early identification and management of complications using simple and convenient tools such as LUS would be valuable. In addition, keeping the ventilated lung stable can be just as important as thoroughly cleaning the non-ventilated lung. Clarifying the relationship between the WLL procedure and worse outcomes may help to identify therapeutic strategies to avoid complications after WLL. Further studies are required to examine this mechanism.

Our case report focused on anesthetic management using LUS to identify intraoperative complications often encountered during WLL. In particular, it is essential to monitor non-ventilated and ventilated lungs using LUS to prevent complications during the procedure. This case provides insight into the procedure and usefulness of LUS from an anesthesiologist’s perspective and potential intraoperative complications. The availability of LUS during the WLL procedure may enable early diagnosis of complications and improve patient prognosis.

### Notes

#### Ethical statements

This study was approved by the Institutional Review Board (IRB) of Ulsan University Hospital (IRB No: 2021-06-020). Written informed consent was obtained for the publication of this case report and accompanying images.

#### Conflicts of interest

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

#### Author contributions

Conceptualization: JWJ, JO; Formal analysis, Funding acquisition, Supervision, Validation: JO; Methodology: HL, JO; Project administration: HL; Writing-original draft: JWJ, HL, JO; Writing-review & editing: JWJ, JO.

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