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IMAGE

Editorial

Ambient Air Pollution: an Important Global Issue

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Abstract

In recent years, climate change and increasing human economic activity has resulted in air pollution becoming an important global issue; air pollution-related health issues are also globally gaining more interest. Air pollution reportedly increases the risk of respiratory, cerebrovascular, cardiovascular, and degenerative diseases (e.g., dementia and osteoporosis) as well as autoimmune disorders. In addition to health issues, human economic activity has led to power shortage. The Taiwan Government needs to develop complete mass transit systems and emission standards to reduce fossil fuel emission and establish standard analytical methods of monitoring air pollutant levels. Because of the antinuclear trend in the global public opinion, the Government needs to immediately consider the use of alternative green energy to address power shortage. Petrochemical industries should improve their processes and replace coal with clean fuel. Quality of the air surrounding petrochemical plants should be measured and monitored. In addition, neighborhood resident health should be followed up over the long term.

Key words: air pollution, cerebrovascular disease, cardiovascular diseases, dementia, osteoporosis

Air pollutants

According to the US Environmental Protection Agency (EPA) criteria, air pollutants include carbon monoxide (CO), nitrogen oxides (NO_x), ground-level ozone (O_3), sulfur oxides (SO_x), particulate matter (PM), and lead (Pb). According to their diameter, PM can be grouped into fine particles (diameter, ≤2.5 μm; $PM_{2.5}$) and coarse particles (diameter, 2.5–10 μm; PM_{10}). Furthermore, several other air pollutants, including acrolein, asbestos, benzene, carbon disulfide, creosote, fuel oils, kerosene, polycyclic aromatic hydrocarbons (PAHs), synthetic vitreous fibers, and total petroleum hydrocarbons, are harmful to health; hence, their levels need to be monitored.[1]

Traffic emissions are the main source of air pollution.[2] Other possible sources include emissions from industries, agriculture, power plants, and coal and other fossil fuel combustion.[3] Several studies have reported that air pollution increases the risk of respiratory, cerebrovascular, cardiovascular, and degenerative diseases (e.g., dementia and osteoporosis) as well as autoimmune disorders.[4-8] The International Agency for Research on Cancer has categorized air pollutants on the basis of carcinogenicity. For example, $PM_{2.5}$, asbestos, benzene, and PAHs belong to group 1, representing agents that are carcinogenic to humans. Creosote belongs to group 2A. Pb and acrolein belong to group 2B and 3, respectively. Group 2 includes agents that are possibly carcinogenic to humans, whereas group 3 includes agents that have not yet been classified in terms of their potential carcinogenicity to humans.[9]

Global Air Pollution

The Governor of Bangkok, Thailand, recently ordered the closure of 437 schools for up to 5 days

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owing to air pollution of a hazardously unhealthy level. Long-term exposure to air pollution caused >4.2 million deaths in 2015.[10] Lelieveld et al. reported 3.3 million premature deaths worldwide due to exposure to ambient air pollution.[11] According to the World Health Organization (WHO), 90% of residents breathe air with high levels of pollutants.[12] In addition, >80% of urban residents in low- to middle-income countries are exposed to air pollution levels greater than those specified as normal in WHO guidelines.[12] Although air pollutant emissions have reduced over the past decades, in 2018, the European Union filed a lawsuit against six member countries accused of failing to control air pollutant emissions. Air pollution is also reportedly the most important environment-related death-causing risk factor.[13]

Air Pollution in Taiwan

The second-largest coal-fired power plant and the largest single-source CO_2 emitter in the world is located in Taichung City, central Taiwan. Therefore, particularly for residents of that area, air quality has always been a matter of public interest. In 2018, in Taiwan's midterm elections, two mayoral candidates from Taichung City debated air pollution, and this issue still troubles the current mayor. In fact, the air pollution levels in Taichung City, particularly of $PM_{2.5}$, are lower than the national average, and Taichung City ranks 12th out of 22 counties and cities in reducing $PM_{2.5}$ emission.[14]

In 2016, the Government of Changhua County, central Taiwan, did not extend the local permit for the Changhua plant of a large chemical corporation because the plant was using bituminous coal with 1.2% sulfur level. This sulfur level was higher than the promised 0.84%–0.87% and, based on air quality deterioration, was deemed capable of causing health problems for residents.

Yuan et al. reported that in adults, exposure to petrochemical complex emission is correlated with PAH emission.[15] A long-term retrospective study identified the occurrence of all types of cancers in residents living near a petrochemical plant in Mailiao Township, Yunlin County, central Taiwan.[16] To protect children, the Executive Yuan of the Taiwan Government demanded that a primary school in Mailiao Township be moved away from the petrochemical plant. In response to this, local residents urged the Yunling County Government to limit permits for the petrochemical plant. In addition to health problems, human economic activity has also led to the problem of power shortage. In Taiwan, thermal power generation by the Taipower system accounts for 84.37% of the total energy production, with nuclear energy accounting for 9.33% (2017).

The Taiwan EPA has established 77 air quality-monitoring stations (AQMSs) across the nation. These AQMSs measure the levels of air pollutants, such as CO, carbon dioxide (CO_2), O_3, nitric oxide (NO), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), $PM_{2.5}$, PM_{10}, methane (CH_4), non-methane hydrocarbons, and total hydrocarbons (THC), over the long term. Because of the high correlation between the air pollutant levels and population density, locations of AQMS were selected on the basis of the urbanization level. There are 32, 15, 22, and 5 AQMSs located in northern, central, southern, and eastern Taiwan, respectively; further, there is one AQMS in each of Taiwan's three outlying islands (Matsu, Kinmen, and Penghu).

Future Work

The AQMS database contains hourly air pollutant levels since January 1, 1998. In 2012, the Taiwan Government passed legislation to measure the $PM_{2.5}$ level. In addition, there are many other harmful air pollutants for which the Taiwan EPA has still not established any standard analytical methods or emission standards. These issues and shortcomings will have to be addressed in future studies. The Government also needs to construct complete mass transit systems and encourage people to use public transportation instead of private vehicles to reduce fossil fuel emissions.

Because of the antinuclear trend in the global public opinion, to address power shortage, the Government needs to immediately consider the use of alternative green energy. Further, the petrochemical industry should improve its processes and replace coal with clean fuel. Measuring and monitoring the quality of air surrounding petrochemical plants should begin immediately. In addition, neighborhood resident health should be followed up over the long term, with emphasis on the cancer incidence and liver-related issues especially in children.

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空氣汙染：一個重要的全球議題

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摘要

隨著氣候變化和人類經濟活動的發展，近年來空氣污染已成為全球關注的重要問題。全球上，相關的健康問題正在受到越來越多的關注。研究證明，暴露於空氣污染中，會增加罹患呼吸道，腦血管和心血管疾病以及退化性疾病（如癡呆和骨質疏鬆症）的風險，並且還會增加自身免疫疾病的發病率。因此政府應建立完整的公共交通系統，建立空氣污染物之標準的分析方法以及空氣汙染物之排放標準。再者，隨著經濟的發展，電力短缺的問題逐漸顯現出來。然而，全球輿論存在反核趨勢，因此，在尋求解決電力短缺問題時，尋求替代性綠色能源需要立即的被關注。

關鍵詞：空氣汙染、腦血管疾病、心血管疾病、失智症、骨質疏鬆症

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Review Article

Role of the Renin–Angiotensin System in PM2.5-Induced Lung Injury: A Mini-review

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Abstract

Air pollution has worsened in recent decades and is known to adversely affect the human respiratory and circulatory systems. Many studies have shown that some constituents of polluted air can trigger or aggravate respiratory and cardiac diseases by inducing lung injury through PM2.5-induced cytokine release and oxidative stress. To review recent findings regarding the effects of fine particles with an aerodynamic diameter of 2.5 μm or less (PM2.5) on lung injury, searches on the Cochrane Library, PubMed, and Google Scholar were performed using keywords "PM2.5," "air pollution," "lung injury," "renin–angiotensin system," and others. The relationships among these effects, including the renin–angiotensin system, and the lack of angiotensin-converting enzyme II were discussed. The involvement of the angiotensin-converting enzyme II–angiotensin-(1-7)–Mas axis in the regulation of inflammatory processes and the context of lung inflammation and fibrosis was reviewed. Recent studies have provided some clarity regarding the mechanisms underlying PM2.5-induced lung injury, which may aid in the development of novel treatments for PM2.5-related pulmonary diseases and injuries.

Key words: Air pollution, PM2.5, renin–angiotensin system, ACE2, lung injury

Introduction

Air pollution has worsened worldwide in recent decades due to the development of heavy industry, increasing usage of fossil fuels, and other factors. Ambient particulate matter has various sources, ranging from natural forest fires to the products of human activities, such as emissions from vehicles, factories, and power plants. Fine particles with a diameter of 2.5 μm or less (PM2.5), which are the main source of air pollution, can adversely affect the human respiratory and circulatory systems[1,2]. Increased concentrations of particulate matter are significantly associated with mortality and certain morbidity endpoints.

Epidemiologic studies have demonstrated that PM2.5 is strongly related to several kinds of respiratory diseases. Molecular and animal studies have revealed the mechanisms of the damaging effects of PM2.5 on the respiratory system and alveolar cells. Among these mechanisms, PM2.5-induced overexpression of transcription factors causing release of inflammatory cytokines has been widely reported. Recent data show that the renin–angiotensin system (RAS) not only affects the cardiovascular and renal systems but also modulates cellular synthesis of cytokines, chemokines, and transcription factors. In this mini-review, we examine existing knowledge regarding the effects of PM2.5 on lung injury and

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the relationships among these effects, the RAS, and angiotensin-converting enzyme II (ACE2).

Relationship between PM2.5 and Lung Injury

Approximately 96% of PM2.5 is retained in the lungs due to the small size of the particles[3]. PM2.5 has various components, including polycyclic aromatic hydrocarbons, oxygenated volatile organic compounds, and heavy metals, which exert toxic effects on humans and animals[4,5]. Inflammation resulting from the excessive inhalation of PM2.5 plays a role in a number of pulmonary diseases, such as acute lung injury, asthma, and chronic obstructive pulmonary disease[6-8]. In addition to the direct harmful effects of PM2.5 deposition on the lungs, several mechanisms of lung injury caused by inhalation of PM2.5 have been widely investigated. Earlier studies showed that the organic components of PM2.5 can induce accumulation of free radicals in the lungs, resulting in the oxidization of alveolar cells. One of the products of oxidization is hydroxyl radicals, which damage cell DNA, resulting in mutagenesis and carcinogenesis[9,10]. In addition, PM2.5-induced inflammatory injury is involved in cytokine release and overexpression of a number of transcription factors. Increased numbers of neutrophils in the blood and of eosinophils, T cells, and mastocytes in the bronchoalveolar fluid in PM2.5-induced inflammation have been reported[11]. The release of numerous cytokines and chemokines, such as interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), and tumor growth factor β1 (TGF-β1), is also related to PM2.5-induced lung injury[12]. These inflammatory cytokines activate the mitogen-activated protein kinase (MAPK) and Janus-activated kinase (JAK) pathways[12-14]. Signal transducer and activator of transcription-3 (STAT3) regulates inflammation and protease expression, which are critical processes in airway injury and lung tissue destruction, and is activated in the lungs of mice exposed to cigarette smoke[15]. Previous studies have shown that matrix metalloproteinases (MMPs) are important factors in lung diseases. Smoke-induced elevation in MMP-10 expression seen in $STAT3^{(+/+)}$ mice was not observed in $STAT3^{(-/-)}$ mice. MMPs are regulated via the pathways of STAT3 and MAPK signaling transducer[15,16], and it is possible that PM2.5-induced activation of the MAPK pathway and its subsequent effects on MMP levels play a role in the mechanisms underlying PM2.5-induced lung injury. Further knowledge of these mechanisms is needed for the effective prevention of PM2.5-related pulmonary diseases.

RAS in lung inflammation

The RAS plays an important role in the regulation of electrolytes and volume homeostasis and blood pressure. Angiotensinogen is cleaved to angiotensin I (Ang I) by renin and is further converted to angiotensin II (Ang II) by angiotensin-converting enzyme (ACE), which is mainly located in the capillaries of the lungs. The RAS has been widely reported to be involved in the pathogenesis and evolution of inflammatory responses. Ang II, a key mediator of inflammation in experimental studies, activates an inflammatory process the upregulation of the synthesis of proinflammatory cytokines and subsequent activation of the nuclear factor (NF)-κB pathway[17-19]. The systemic infusion of Ang II into normal rats increases renal NF-κB and activator protein 1 binding activity, which is associated with infiltration of inflammatory cells[20].

In addition to inflammation in other parts of the body, the RAS is involved in the pathogenesis of lung inflammation, with expression of the RAS components and increased levels of ACE seen in various interstitial lung diseases and in bronchoalveolar fluid in fibrotic lung disease[21]. It has been speculated that the pulmonary RAS, when activated, may promote the development of lung diseases and injuries through a variety of effects at the cellular level, such as alteration in vascular tone, vascular permeability, and fibroblast activity. This speculation is based on the observation that angiotensinogen and Ang II are increased in patients with lung disease and in animal models[22,23], indicating the existence of a pulmonary RAS and the possibility that the end product, Ang II, may play a significant role in lung injury. Ang II is the main effector in the RAS cascade that has biological activity. Its level is increased in the lungs of normal rats following injury as a result of elevated angiotensinogen expression in injured pulmonary epithelial cells and radiation-induced pulmonary fibrosis[24,25]. Over-distension of the alveoli through mechanical ventilation with high tidal volume results in the activation of the inflammatory process in male

rats, leading to ventilator-induced lung injury. The expression of RAS components in rat lung tissue, including angiotensin and Ang II, is increased[26]. Moreover, ACE, which is the other component of the RAS, aggravates the pathogenesis of pulmonary disease, induces lung edema, and impairs lung function. An animal study showed that pulmonary disease was markedly improved in mice deficient in the ACE gene[27]. Another study showed that pretreatment with an ACE inhibitor decreased lung inflammation by suppressing TNF-α, NF-**κ**B activity, and blood levels of Ang II [28].

ACE2–Ang-(1-7)–Mas axis in PM2.5-related lung inflammation

As noted above, PM2.5 may include components, such as heavy metals and organic substances, which can trigger the release of several cytokines, leading to inflammation and injury of lung tissue by activation of inflammatory pathways, including JAK–STAT and MAPK. The increased expression of angiotensinogen, renin, and ACE in multiple tissues led to the suggestion that multiple local RASs may act independently of the systemic RAS[29,30]. Among the multiple local RASs, pulmonary RAS is involved in the regulation of lung inflammation. Inappropriate decreased local expression of Ang II can result in tissue damage and chronic injury. Previous studies have shown that the JAK–STAT pathway mediates Ang II-triggered gene transcription and that the JAK–STAT pathway may act as an amplifying system to further activate local RASs. These findings suggest that the JAK–STAT pathway has an important role in the mechanism of RAS-associated tissue injury[31].

In recent decades, other components of the RAS, including (pro)renin receptor, ACE2, and other angiotensin peptides and receptors, have been widely discussed. The decapeptide Ang I is cleaved to the octapeptide Ang II by ACE. ACE2 converts Ang I into angiotensin-(1-9) (Ang-(1-9)) and cleaves a residue from Ang II to produce angiotensin-(1-7) (Ang-(1-7)). ACE2 was less discussed before 2003, when ACE2 was found to be a functional receptor for severe acute respiratory syndrome coronavirus. Reduction in the normal levels of ACE2 was shown to improve resistance to inflammatory lung diseases[32]. Increased expression of Ang II triggered by ACE results in severe lung injury through the Ang II type 1 receptor-a receptor. Possible effects include induction of pulmonary vasoconstriction, increased vascular permeability, upregulation of pulmonary inflammatory cytokines, acceleration of Fas-induced apoptosis in alveolar epithelial cells, and promotion of extracellular matrix synthesis and lung fibroproliferation[24]. These findings provide more evidence that the role of the RAS is not limited to the cardiovascular system and that components in addition to Ang II, including Ang-(1-7) and Ang-(1-9), are involved in many actions in addition to blood pressure control[33,34]. ACE2, as an RAS antagonist, has greater catalytic efficiency in the hydrolysis of Ang II to Ang-(1-7) than in the hydrolysis of Ang I to Ang-(1-9)[35]. Systemic infusion of Ang II into rats resulted in the elevation of renal expression of TNF-α and also upregulated proinflammatory mediators, including IL-6, monocyte chemoattractant protein 1, and NF-kβ[18]. A rat model of autoimmune myocarditis showed that angiotensin receptor blockers increased ACE2, Ang-(1-7), and Mas expression and decreased expression of proinflammatory cytokines, such as TNF-α, interferon γ, interleukin-1 β, and IL-6[36,37]. In a pancreatitis cell model (caerulein-treated pancreatic acinar cells), exogenous Ang-(1-7) decreased the expression of IL-6 and TNF-α mRNA, suggesting that the ACE2–Ang-(1-7)–Mas axis suppresses the production of inflammatory factors[38]. An animal study showed that the loss of ACE2 expression resulted in increased pulmonary vascular permeability as blue dye accumulation was greatly increased after acid aspiration in ACE2–Ang-(1-7)–Mas (ACE2 KO) mice. The symptoms of acute lung injury were attenuated by treatment with active recombinant ACE2 protein[27]. Therefore, the ACE–Ang II–AT1 receptor axis is responsible for triggering lung injury, whereas ACE2, which acts as a negative regulator of the RAS by inactivating Ang II, plays a protective role in acute lung injury. Moreover, its product, Ang-(1-7), exerts antiapoptotic, anti-inflammatory, and antifibrotic effects through the ACE2–Ang-(1-7)–Mas axis[39].

PM2.5 may cause lung injury through JAK–STAT or MAPK inflammatory pathways, which are activated by inflammatory cytokines, such as IL-6, TNF-α, TGF-β1, and others, and the RAS is involved in the mechanism of local or systemic inflammatory response. Thus, we may deduce that the RAS may have an important role in PM2.5-induced lung injury and inflammation. However, few studies have

focused on the relationships between PM2.5 and the RAS or ACE2. When we challenged the effect of ACE2 on bleomycin-induced pulmonary injury, the levels of IL-6 and TGF-β1 in the lungs of ACE2 KO mice were significantly higher than those in wild-type mice administered intratracheally, which indicated that ACE2 deficiency may exacerbate bleomycin-induced lung injury[40].

A recent study sought to address this research gap by investigating the role of ACE2 in PM2.5-induced acute lung injury[41]. The study utilized an animal model of PM2.5-induced acute lung injury through the production of wild-type and ACE2 KO mice. The results show that the respiratory rates as well as the inflammatory cytokine, ACE, and MMP levels in the lungs of the wild-type and ACE2 KO mice were significantly increased on the second day after acute instillation of PM2.5. At 5 days postinstillation, however, the wild-type mice had significantly recovered from the PM2.5-induced lung injury, whereas the ACE2 KO mice exhibited only partial recovery. These results clearly indicate that the deficiency in ACE2 of the ACE2 KO mice led to attenuation of injury repair, enhancement of the inflammatory response, and tissue remodeling in response to PM2.5 instillation, further clarifying the relationships among the RAS, ACE2, and PM2.5 intake.

Conclusion

A number of studies have illuminated the role of PM2.5 air pollution in lung injury and lung inflammation, as well as the related effects of the RAS and ACE2. ACE2 may have a protective effect in PM2.5-induced lung injury as a result of abnormal accumulation of Ang II. More evidence is needed regarding the underlying mechanism of PM2.5-induced lung injury to provide effective prevention of deleterious PM2.5-induced pulmonary effects.

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腎素 - 血管收縮素系統在空氣汙染物 PM2.5 引發肺損傷中的角色：一個小型綜論

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摘要

背景：近幾十年來普遍惡化的空氣汙染已是被公認為造成呼吸道及循環系統問題的主要原因 。
目的：過去的研究已經發現粒徑小於或等於 2.5μm 的懸浮微粒和肺損傷有莫大的相關性 ，而可能的原因又與腎素 - 血管收縮素系統及缺乏第二型血管收縮素轉化酶有關 。
方法：文獻回顧的方式採用搜尋 Cochrane Library, PubMed, and Google Scholar 等系統的方式 ，搜尋地的關鍵字為空氣汙染 、PM2.5 肺損傷及腎素 - 血管收縮素系統等 。
主要結果與結論：現存的文獻明確的提供一些有關腎素 - 血管收縮素系統在 PM2.5 導致肺損傷中所扮演的角色及機制 ，希望藉由這些證據有助於發展針對 PM2.5 有關的肺病與肺損傷的全新的療法 。

關鍵詞：空氣汙染 、PM2.5 、腎素 - 血管收縮素系統 、血管收縮素轉化酶 2 、肺損傷

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Original Article

Effects of Hypothermia on the Viability of A549 Cells Exposed to Hydrogen Peroxide and Liposaccharide

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Abstract

Background and purpose: Therapeutic hypothermia has been demonstrated to protect brain tissue after hypoxic brain injury, but its influence on injured pulmonary cells is questionable. In sepsis-induced acute respiratory distress syndrome, oxidative stress and endotoxin-induced severe pulmonary inflammation promote lung injury. Hypothermia may be beneficial to alleviate injury to alveolar cells.

Methods: Human alveolar epithelial cells (A549) were cultured at 37°C (normothermia) or 34°C (hypothermia) and then stimulated with lipopolysaccharide (LPS) (10 μg/mL), hydrogen peroxide (H_2O_2) (10 μM), or both (LPS+H_2O_2) for 6 to 48 h at 37°C (normothermia). To compare the effects of hypothermia, A549 cells were stimulated with H_2O_2, LPS, or both, and cultured at 34°C or 37°C for 24 h. Non-stimulated cells incubated at the same temperature and duration were used as the control group. Cell viability was determined using the MTT assay.

Results: Stimulation with H_2O_2 or LPS+H_2O_2 at 37°C for 6 to 48 h quickly and significantly decreased cell viability to less than 20% of that of non-stimulated cells. The viability of A549 cells stimulated with H_2O_2 vs. LPS + H_2O_2 under hypothermic conditions (34°C) for 24 h was significantly greater than at 37°C (19 ± 18% vs. 4 ± 3% and 22 ± 21% vs. 3 ± 1%, respectively, $p < 0.05$).

Conclusions: Hypothermia at 34°C significantly protected A549 cells from H_2O_2- or LPS+H_2O_2-induced death. Further studies are required to clarify the role of hypothermia in injured pulmonary tissues.

Keywords: acute lung injury, hypothermia, lipopolysaccharide, human alveolar epithelial cell, hydrogen peroxide

Introduction

Therapeutic hypothermia (TH) has been used to protect tissues after cardiac arrest or traumatic brain injury[1]. The key aspects of TH are the potential abilities to diminish cellular metabolism and reduce the demand for oxygen and glucose[2,3]. Previous studies have found that TH may also affect the apoptotic pathway and prevent stress-induced cell death[4-6]. Moreover, TH has been widely applied in neonatal critical care to reduce brain damage of neonates caused by birth asphyxia[7,8]. Current protocols recommend starting TH within the first 6 h of life with cooling to either 33.5 ± 0.5°C for whole-body cooling or 34.5 ± 0.5°C for head cooling, and continuing hypothermia for 48 to 72 h[9].

The influence of extended TH on organs other than the brain remains unclear, thus further investigations are warranted. Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are major causes of mortality of critical patients. Patients that develop acute diffuse injury of lungs often have underlying diseases, such as sepsis[10,11]. In ARDS, severe pulmonary inflammation may increase

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endothelial and epithelial permeability, and the loss of aerated lung tissue, resulting in poor gas exchange and diffuse radiographic opacities on chest X-rays[11]. It has been reported that hypothermia attenuated ventilation-induced alveolar epithelial injuries and preserved the mechanical properties of the lung[12]. Therefore, hypothermia may be beneficial for the treatment of ALI.

Systemic inflammatory responses resulting from severe infections can cause sepsis. In sepsis-induced ARDS, the major mechanisms are considered to be related to the release of inflammatory mediators due to sepsis. These inflammatory mediators can be released into the systemic circulation and subsequently induce various cellular and molecular responses that affect distal organs, including the lungs[13]. Therefore, hypothermia has the potential to decrease inflammatory responses in sepsis, suggesting a potential benefit for the treatment of sepsis-induced ARDS.

Previous studies have reported that hypothermic conditions may lower the risk of oxidative stress-induced cell damage and programmed cell death by upregulating the expression of the anti-apoptotic protein *bcl*-2 [5], which helps to preserve the mitochondria and conserve DNA integrity [14]. However, past studies have rarely focused on pulmonary tissues. Therefore, further studies are necessary to elucidate the effects of hypothermia on alveolar epithelial cells with or without injury.

Lipopolysaccharide (LPS), a component of the gram-negative bacterial cell wall, is commonly used to induce severe inflammatory responses and immune system dysfunction[15]. Many inflammatory mediators released into the systemic circulation after LPS stimulation induce cellular and molecular responses of the brain and lung tissues[16]. Intratracheal administration of LPS has been used as a clinically relevant animal model of severe lung injury[15,17,18]. Also, LPS-induced alveolar cell damage has been used to explore cellular events in sepsis-induced ARDS[15-21]. In addition, reactive oxygen species (ROS) are known to play an important role in the pathogenesis of ARDS[22,23] and high concentrations of hydrogen peroxide (H_2O_2) in breath condensate have been reported in patients with ARDS[24]. H_2O_2 is commonly used to experimentally investigate the effects of ROS in alveolar epithelial injury[4,25]. Therefore, the use if an *in vitro* model may be suitable to test the effects of hypothermia on alveolar epithelial cells mimicking sepsis-related ARDS following administration of LPS and H_2O_2.

We hypothesized that hypothermia may be beneficial to defend against injury to alveolar epithelial lung cells and prevent inflammatory mediator release caused by LPS- or H_2O_2-induced oxidative stress. Therefore, the purpose of this study was to investigate the *in vitro* protective effects of hypothermia on LPS or H_2O_2-induced alveolar epithelial injury.

Materials and Methods

Human type II alveolar cell culture

Commercial human type II alveolar cells (A549), a human lung adenocarcinoma epithelial cell line that retains many characteristics of type II alveolar cells from the pulmonary epithelium[4], were purchased from the Bioresource Collection and Research Center (BCRC 60074; Hsinchu, Taiwan) and maintained in F-12K medium (F-12K Nutrient Mixture, Kaighn's modification; Invitrogen Corporation, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 0.25 μg/mL of amphotericin B at 37°C under an atmosphere of 5% CO_2/95% air. The culture medium was replaced every 2–3 days during the experiment[4].

The A549 cells were plated into the wells of 24-well culture plates at a concentration of 1×10^6 cells/mL (0.9 mL/well) and cultured overnight in serum-free F12K medium.

LPS stimulation

The A549 cells were cultured with LPS [100 μg in 100 μL of phosphate-buffered saline (PBS); final concentration, 10 μg/mL; 0.9 mL/well)] at 34°C for 24 h for stimulation or with 100 μL of PBS at the same temperature and duration as a control group.

H_2O_2 stimulation

The H_2O_2 concentration was diluted from 10 M (30% solution) to 100 μM with PBS for the following experiments. The A549 cells were cultured in 100 μL of 100 μM H_2O_2 (final concentration, 10 μM; 0.9 mL/well) at 34°C for 24 h for stimulation or with 100 μL of PBS at the same temperature and duration as a control group.

Cell viability assay

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma–Aldrich Co., St. Louis, MO, USA) was performed to assess cell viability as described in our previous report[4]. Briefly, A549 cells were seeded into the wells of 24-well plates (1×10^6 cells/mL, 0.9 mL/well). After exposure to LPS or H_2O_2 to mimic normo- or hypothermia conditions, the cells were washed twice with 1 mL of PBS and then incubated with 1 mL of MTT (0.5 mg/mL) for 4 h at 37°C. Afterward, the medium was aspirated and the cells washed with PBS again. The formazan product was dissolved in 0.5 mL of dimethyl sulfoxide and the optical density at 570 nm was determined using a microplate spectrophotometer (Sunrise Absorbance Reader; Tecan Austria GmbH, Salzburg, Austria). The optical density of a blank well was subtracted before data analysis[4].

Statistical analysis

Data are expressed as the mean ± standard deviation. Data comparisons between two groups and among three groups were conducted using the *t* test and one-way analysis of variance, respectively. The *post-hoc* Student–Newman–Keuls test was used for pairwise comparisons. A probability (*p*) value of < 0.05 was considered statistically significant.

Results

The experimental A549 cells were the squamous type and grew well in F12K medium (Fig. 1A and B). After H_2O_2 stimulation, marked cell death was observed within 6 to 48 h (Fig. 1C and D). The viabilities of A549 cells exposed to H_2O_2, LPS or H_2O_2+LPS for 6 and 48 h are summarized in Fig. 2. As shown, marked cell death was observed in the both H_2O_2 and H_2O_2+LPS groups from approximately 15% at 6 h to 5% at 24 h. However, there was no further decrease between 24 to 48 h and there was no significant difference in cell viability between the H_2O_2 and H_2O_2+LPS groups at any time point. Notably, there was no difference in cell viability between the LPS (10 μg/mL) and control groups.

Cell viability at 37°C and 34°C after stimulation

The results of comparisons among the different groups at 37°C and 34°C are shown in Fig. 3. As shown,

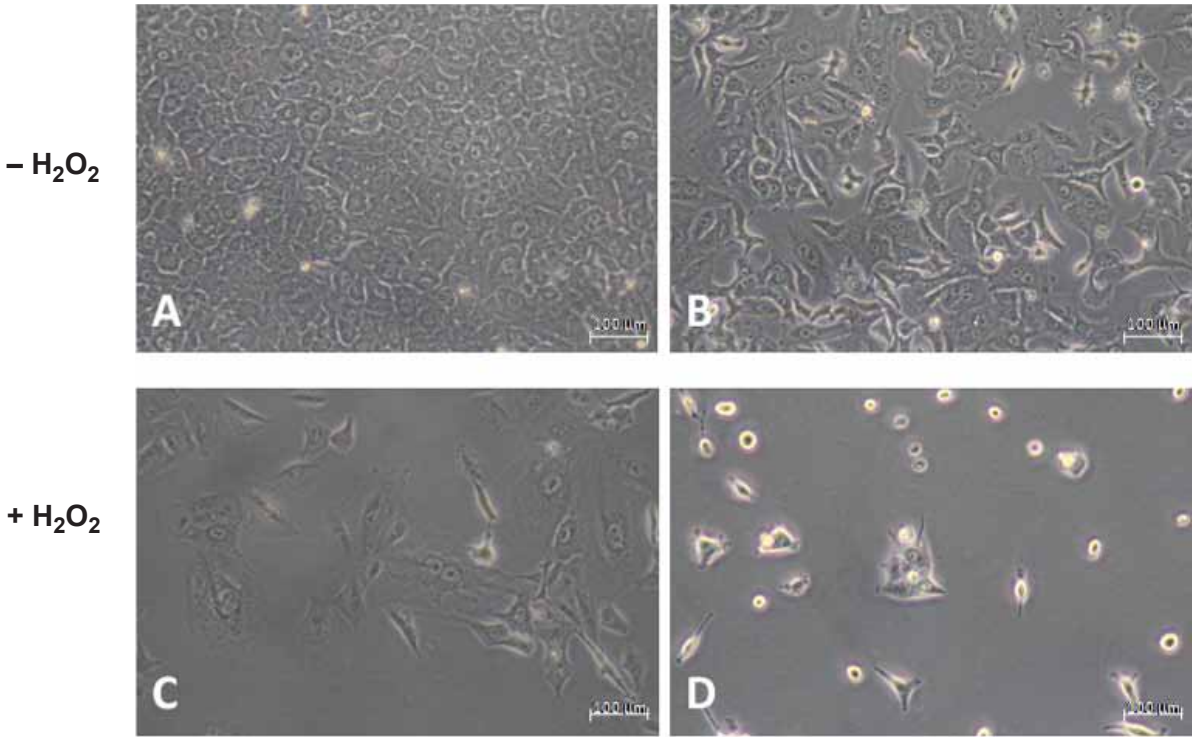

Fig. 1 Microscopic appearance of experimental A549 cells with or without H_2O_2-induced injury. (A) Non-injured fully grown cells. (B) Non-injured cells at baseline status (1×10^6/μL). (C) Some cells survived after injury for 24 h (D) Cells rarely survived after exposeure to H_2O_2 for 24 h.

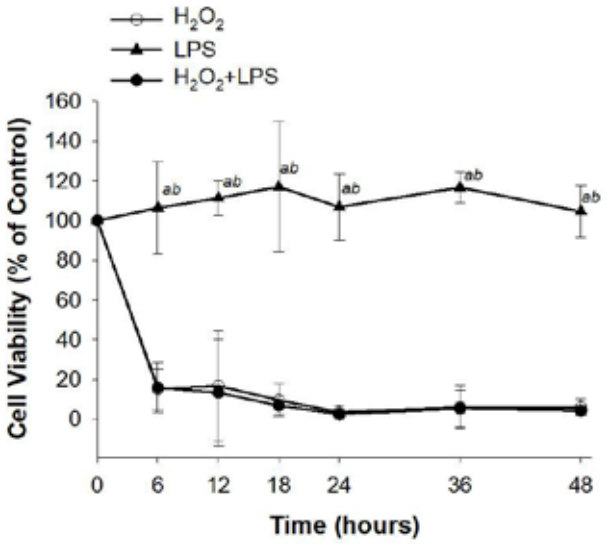

Fig. 2 Viability of A549 cells stimulated with H_2O_2, LPS, or H_2O_2 + LPS at 37°C. Data are presented as the mean ± standard deviation (n = 8/group). $^{a}p < 0.05$ vs. H_2O_2 group and $^{b}p < 0.05$ vs. LPS group at the same time point.

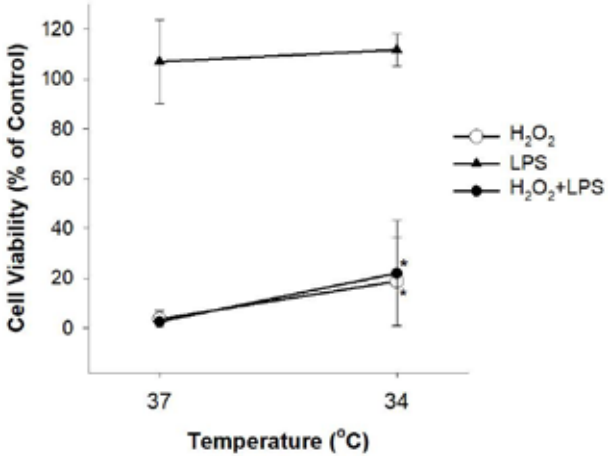

Fig. 3 Cell viability between groups at 37°C and 34°C. Data are presented as the percentage of non-injured cells. $^{*}p < 0.05$ of the same injury group at 37°C.

cell viability of the H_2O_2 and H_2O_2+LPS groups was enhanced from 4 ± 3% and 3 ± 1% at 37°C to 19 ± 18% and 22 ± 21% at 34°C, respectively ($p < 0.05$). In the LPS group, there was a slight increase in cell viability at 34°C (107 ± 17%), as comparison to 37°C (117 ± 6%), but this difference was not significant ($p > 0.05$).

Discussion

The results of this *in vitro* study demonstrated the protective effects of hypothermia on the viability of alveolar epithelial cells exposed to LPS+H_2O_2 or H_2O_2 only.

TH lowers body temperature, thereby protecting the brain and lung tissues by decreasing general inflammatory responses to septic conditions. However, Sarkar et al. found no difference in gas exchange rates or pulmonary mechanics by the application of hypothermic conditions to asphyxiated newborns without lung injury[26]. Additionally, Lim et al. demonstrated that TH could decrease vascular manifestations and the extent of alveolar epithelial injury in an animal model of AKI[12]. Our previous study also supported the protective role of TH to prevent further damage to H_2O_2-injured rather than uninjured alveolar epithelial cells. Nevertheless, we also observed that hypothermia itself caused an increase in the rate of apoptosis and increased intracellular ROS levels in uninjured cells[4].

To mimic the conditions of sepsis, intratracheal administration of LPS, a component of the gram-negative bacterial cell wall, is commonly used to experimentally induce severe inflammatory responses and immune system dysfunction in animal models[15,17,18,27]. LPS-induced alveolar cell damage has also been used to investigate cellular conditions in sepsis-induced ARDS[15,16,19-21]. Rodriguez-Gonzalez et al. reported that LPS-stimulated A549 cells released inflammatory mediators that induced harmful responses in rat brain cells[16].

In the present study, LPS as well as H_2O_2 were used to mimic sepsis-related ARDS by inducing damage to lung cells. The results of our *in vitro* study showed LPS at a concentration of 10 μg/mL did not significantly increase apoptosis of A549 cells, in contrast to the finding of other reports[15,21,28,29]. A possible explanation for these conflicting findings may be the induction of systemic inflammatory responses in response to bacteria endotoxins, suggesting an incomplete LPS-mediated influence on alveolar cells due to differences in the experimental parameters, especially the dosage of LPS, among the studies. In many published reports, LPS concentrations of 0.1 to 10 μg/mL were most commonly used to induce injury to A549 cells[15,16,19,21,29]. Zhao et al. reported that median lethal dose of LPS to A549 cells was 1 μg/mL for 24 h[19], while other investigators reported significant A549 cell injury at 10 μg/mL[15,21,29]. Even in the absence of cell death, inflammatory factors are still produced. Rodriguez-Gonzalez reported that 0.1 μg/mL of LPS was sufficient to stimulate A549 cells

without causing significant cell death[16] and Nishio et al. reported that cell death was insignificant at LPS concentrations of >0.5 mg/mL[28]. Theoretically, higher dosages of LPS will cause more significant cell injury. Although there was no significant cell death at LPS concentrations of >10 μg/mL in the present study, H_2O_2 administration induced severe oxidative stress, which resulted in significantly increased rates of cell death. Therefore, an LPS concentration of 10 μg/mL was chosen to induce hypothermic effects in our experimental setting. Nonetheless, further studies using higher doses of LPS or with lung tissues in an *in vivo* model are warranted.

The mechanisms underlying the protective effects of hypothermia at 34°C on the survival of injured cells can be explained by the reduced apoptosis rate and decrease in oxidative stress, as demonstrated by Chiou et al.[4].

Although commonly used for *in vitro* studies of lung cell function, A549 adenocarcinomic human alveolar basal epithelial cells, as used in the present study, differ from normal lung cells[4,21,29-31]. Therefore, the use of normal lung cells and primary lung cell cultures should be considered for future *in vivo* studies.

The limitations of the present study include the *in vitro* experimental design, relatively low LPS dosage, limited observation period of only 24–48 h, and the sole focus on cell viability. Since an *in vitro* design is insufficient to replicate whole body responses to LPS, it was not possible to investigate the influence of LPS-induced inflammatory mediators released from other cells. Although various LPS concentrations to induce cell death have been reported, higher dosages should be considered in future experiments. Lastly, the current study was designed simply to test cell viability, thus further designs will be necessary to identify other cellular protective mechanisms induced by hypothermia.

In conclusion, exposure of alveolar epithelial A549 cells to 10 μM of H_2O_2 for 6 to 48 h rather than 10 μg/mL of LPS was sufficient to induce significant cell death in this *in vitro* study. Moderate hypothermia at a temperature of 34°C had a significant protective role in A549 cells exposed to H_2O_2 or H_2O_2 + LPS. Further studies are required to clarify the role and mechanisms of hypothermia in injured pulmonary tissues.

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低溫對受過氧化氫與脂多醣刺激後的肺細胞存活率的影響

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摘要

背景及目的：低溫療法被認為可有效保護缺氧後的腦細胞，但是低溫療法對肺泡細胞病變的影響仍有待研究。在敗血症引起的急性呼吸窘迫症候群時，會引發許多發炎介質的分泌而傷害到肺組織。治療性低溫療法對敗血症引起的肺組織傷害可能有保護效果。本研究目的是探討低溫對模擬敗血症引起的急性呼吸窘迫症候群的受損肺泡細胞存活率的影響。

方法：本研究以體外細胞研究模式探討低溫療法對脂多醣（Lipopolysaccharide, LPS）與過氧化氫（Hydrogen peroxide, H_2O_2）引起的直接肺細胞傷害的影響。我們將人類肺泡上皮細胞（A549）在 LPS（10 mg/mL）、H_2O_2（10 mM）或 LPS+H_2O_2 刺激後於 37°C 的溫度培養 6、12、18、24、36 與 48 小時，也將 A549 細胞於刺激後培養於低溫中（34°C），分別以 LPS、H_2O_2、或 LPS+H_2O_2 刺激 24 小時，其後做細胞存活率分析。

結果：A549 細胞受 H_2O_2、或 LPS+H_2O_2 刺激後的細胞存活率會快速且顯著下降至 20% 以下。在低溫 34°C 下，受 H_2O_2 或 LPS+H_2O_2 刺激的細胞之存活率（H_2O_2：19 ± 18%；LPS+H_2O_2：22 ± 21%）顯著地比在 37°C 培養的狀態要高（H_2O_2: 4 ± 3%；LPS+H_2O_2：3 ± 1%）（$p < 0.05$）。

結論：在 LPS+H_2O_2 或 H_2O_2 刺激 6 至 48 小時下，肺泡細胞存活率顯著下降。34°C 的低溫培養對暴露於 LPS+H_2O_2 或只有 H_2O_2 的肺泡細胞有保護效果，可減少細胞死亡。相關機轉與臨床應用仍需進一步研究。

關鍵詞：低溫療法、肺泡細胞、急性肺損傷、脂多醣

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Original Article

Clinical Characteristics of Anti-N-Methyl-D-Aspartate Receptor Encephalitis in Children in Taiwan

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Abstract

Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis is a potentially life-threatening disease that has a high probability for recovery if patients are diagnosed early and treated appropriately. As this disease is known for its variable clinical presentations and is relatively unfamiliar to the majority of clinicians, the systemic analysis of the clinical characteristics of patients presented here may assist first-line clinicians in understanding this disease. We conducted a retrospective analysis of patients admitted to the Tungs' Taichung MetroHarbor Hospital with anti-NMDA receptor encephalitis between 2007 and 2015. Their presenting symptoms, clinical features, laboratory and neuroimaging findings, and treatment outcomes were analyzed. Diagnosis was confirmed by the presence of anti-NMDA receptor antibodies in the cerebrospinal fluid or serum in addition to clinical manifestations, whereas probable diagnosis was made by clinical manifestations and laboratory results. The results identified seven patients (male: female: 1: 6) aged between 4 and 13 years old with anti-NMDA receptor encephalitis. Of these patients, 85.7% (6/7) presented with mood, behavioral or personality changes; 100% (7/7) had orofacial dyskinesia, 57.1% (4/7) experienced seizures, none had an underlying tumor, and 57.1% (4/7) of the patients received intravenous immunoglobulin (IVIG) injection. Overall, 57.1% (4/7) of all patients made a substantial recovery with or without IVIG treatment. In conclusion, anti-NMDA receptor encephalitis is a treatable disease if the diagnosis is confirmed early. The majority of the patients with this disease in the present study presented with psychosis, whereas orofacial dyskinesia was the prominent feature of patients with the disease. IVIG treatment did not guarantee a favorable prognosis; spontaneous recovery may occur in certain patients without IVIG treatment.

Keywords: Anti-N-methyl-D-aspartate (NMDA) receptor encephalitis, orofacial dyskinesia, intravenous immunoglobulin (IVIG)

Introduction

The presentation of anti-N-methyl-D-aspartate (NMDA) receptor encephalitis includes a predictable set of symptoms that combine to form a characteristic syndrome. The clinical course of the condition is separated into multiple phases: (a) prodromal phase, (b) psychotic/seizure phase, (c) unresponsive phase, (d) hyperkinetic phase, and (e) gradual recovery phase.[1] The diagnosis of anti-NMDA receptor encephalitis is confirmed by the detection of immunoglobulin G (IgG) antibodies to the GluN1 subunit of the NMDA receptor on analysis of serum or cerebrospinal fluid (CSF)[2], in addition to the rapid onset of clinical symptoms[3]. Although the incidence of anti-NMDA receptor encephalitis is increasing worldwide, the majority of clinicians remain unfamiliar with this disease. The systemic analysis of local patients with the disease presented here may assist clinicians in understanding this life-threatening but curable disease.

Materials and Methods

Patients admitted to Tungs' Taichung MetroHarbor Hospital (TTMH) due to unknown encepha-

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litis between January 1, 2007 and December 31, 2015 were enrolled in this study, which was approved by the TTMH Institutional Review Board (No. 105045).

Table 1. Summary of the Clinical Features of the 7 Patients with Anti-NMDA-Receptor Encephalitis in Taiwan

Characteristic	Children (n = 7)	Percentage (%)
Prodromal phase		
Fever(38°C)	2	28.6%
Headache and/or dizziness	1	14.3%
Upper respiratory symptoms	3	42.9%
Nausea and/or vomiting	1	14.3%
Psychotic/seizure phase		
Psychiatric symptoms	7	100%
Mood, behavior, or personality changes	6	85.7%
Hallucinations (visual or auditory)	2	28.6%
Neurological symptoms	6	85.7%
Seizures (generalized clonic-tonic)	4	57.1%
Unresponsive phase		
Consciousness disturbance	5	71.4%
Mutism	3	42.9%
Hyperkinetic phase		
Orofacial dyskinesia	7	100%
Temporary dystonic postures	7	100%
Autonomic disturbance	6	85.7%
Recovery phase (Outcome)		
Full	0	0%
Substantial	4	57.1%
Limited	3	42.9%

Abbreviations, NMDA= N-methyl-D-aspartate

Diagnosis was confirmed by (1) the detection of anti-NMDA receptor antibodies in the cerebrospinal fluid, and (2) by the presence of relevant clinical features, including psychiatric symptoms/cognitive dysfunction, speech dysfunction, seizures, movement disorder/dyskinesias/abnormal postures, decreased level of consciousness, autonomic dysfunction, and/or central hypoventilation. The antibodies were sent to a laboratory at the Hospital Clinic of Barcelona in Barcelona, Spain, which specializes in examining the pathogenesis of immune-mediated neuronal disorders. All patients were screened for potential tumors, including tumors in their abdominal and genital regions. The clinical information of each patient, including the patient's age, gender, prodromal symptoms, presentation, serum and CSF biochemical data, bacterial and viral culture data, brain magnetic resonance imaging (MRI), electroencephalograms (EEG), anti-NMDA receptor antibody results, therapeutic agents, clinical responses before and after treatment, and outcomes over 1–3 years of follow-up, were collected.

Results

Clinical Features

Seven cases are summarized in Table 1 and Table

Table 2. Ancillary Tests, Treatments, and Outcomes of the 7 Patients with Anti-NMDA-Receptor Encephalitis in Taiwan

Sex/ Age, year	Ab+	CSF Findings			Tumor	Brain MRI	EEG	Diagnosis time	Treatment	Outcome (Follow-up Time)
		Leu	TP	OCB						
F/5	Not exam	1	38	-	Negative	Negative	Generalized ED	22d	IVIG and pulse steroid	Substantial improvement (3 yr)
M/4	+	3	14	-	Negative	Negative	Focal ED	1m, 13d	AED	Substantial improvement (2.5 yr)
F/4	–	2	23	-	Negative	Negative	Generalized ED	1m, 8d	IVIG and pulse steroid	Limited improvement (1 yr)
F/5	+	1	22	-	Negative	Negative	Focal ED	2m	IVIG and pulse steroid	Substantial improvement (3 yr)
F/5	Not exam	5	33	-	Negative	Negative	Slow background	1y	AED	Limited improvement (2 yr)
F/13	–	1	42	-	Negative	Global involvement as encephalitis.	Focal ED	6d	AED	Substantial improvement (1 yr)
F/7	+	16	56	-	Benign ovarian cyst, right	Negative	Slow wave	1m, 16d	IVIG and pulse steroid	Limited improvement (3 yr)

Abbreviations
AED= Antiepileptic drug, CSF= Cerebrospinal fluid, ED= Epileptiform discharge,
EEG= Electroencephalography, IVIG= Intravenous immunoglobulin, Lym= Lymphocytes,
MP= Methylprednisolone, MRI= Magnetic resonance imaging, OCB= Oligoclonal band,
TP= Total protein , mg/dL
Leu= Leukocytes, Cells/μL

2. The ages of these patients ranged between 4 and 13 years old (median age: 6.1 years); one patient was male and the remaining six were female. In the prodromal phase, 42.9% of the patients exhibited upper respiratory symptoms. In the psychotic/seizure phase, psychiatric symptoms were recorded in all patients, which included mood, behavior, or personality changes (6/7; 85.7%), or hallucinations (2/7; 28.6%). Among neurologic symptoms, generalized clonic-tonic seizures were the most common type (57.1%). In the unresponsive phase, consciousness disturbance was recorded in five patients and mutism was recorded in three patients. In the hyperkinetic phase, orofacial dyskinesia and dystonic postures were frequently noted in the patients. In addition, autonomic disturbance was present in 6/7 patients (85.7%). In the recovery phase, none of the patients made a full recovery. Rather, 4/7 (57.1%) and 3/7 (42.9%) patients made a substantial and limited recovery, respectively.

Laboratory, EEG, and neuroimaging findings

Only 5/7 patients were tested for the anti-NMDA receptor antibody, 3/5 patients were positive for anti-NMDA receptor antibody, whereas 2/5 patients were negative for anti-NMDA receptor antibody and were diagnosed clinically. The majority of the brain MRI findings were negative, and only one case showed global involvement, which was not specific. The EEG findings included the presence of generalized epileptiform discharge (28.6%) or focal epileptiform discharge (42.9%). The median CSF white blood cell count of the patients was 4.1/μl, and the median CSF protein and glucose concentrations were generally within normal limits. None of the patients (0/7) were found to have underlying tumors in the initial survey or in the following years (median 2 years), although one patient was found to have benign ovarian cysts.

Diagnoses, treatments, and outcomes

All of the cases were diagnosed within 1 year from the onset of psychoneurological symptoms (6/7 cases were diagnosed in <2 months). In terms of treatment, 4/7 patients (57.1%) received intravenous immunoglobulin (IVIG), with two of these patients experiencing substantial improvement and the other two experiencing limited improvement.

The remaining three patients (42.9%) received anticonvulsant treatment, two of which experienced substantial improvement and the remaining patient experienced limited improvement. Overall, 4/7 (57.1%) of the patients made a substantial recovery with or without IVIG, and 42.9% (3/7) of the patients made a limited recovery.

Discussion

Anti-NMDA receptor encephalitis was previously documented predominantly in young females with an ovarian teratoma, but an increasing number of cases involving male patients and children have been reported in recent years.[4,5] In one series of 44 patients, 30% were male and 23% were children.[6] In a study of 12 patients in Taiwan by Lin et al., 83.3% were female and 50% were <18 years old.[1] In the present study, six (85.7%) patients were female, and none of the patients had underlying tumors, with the exception of one with a benign ovarian cyst. These results are consistent with the previous studies showing female predominance.

The clinical presentation of anti-NMDA receptor encephalitis usually evolves over five stages.[1] A previous study involving a large series of young adults demonstrated 70% of patients presented with psychiatric symptoms.[5] In the study by Lin et al[1], initial presentation of seizures prior to the onset of psychotic symptoms was predominantly observed in children. In the present study, mood and behavioral changes occurred in the majority of patients (85.7%), with seizures occurring in over half of the patients (57.1%) and orofacial dyskinesia/temporary dystonic postures present in all of the patients (100%). Psychiatric changes and orofacial dyskinesia were more commonly reported in the present study. Therefore, these symptoms may assist pediatricians in making an appropriate diagnosis. In young children, psychiatric symptoms, including behavioral changes, can be difficult to recognize.[2] This can present a challenge for clinicians and a delay in diagnosis is a common feature of the disease. Furthermore, antiepileptic drugs are used to treat the neurological symptoms of the condition. However, abnormal seizure attacks as a recurrence of partial epileptic seizures may lead to the escalation of antiepileptic medications.[2,9]

In the present study, IgG anti-GluN1 antibodies were detected in 3/7 patients; 2/7 patients were negative for the antibodies and antibody test results were not available in the remaining two patients.

The diagnosis of definite anti-NMDA-receptor

encephalitis depended on the results of autoantibody tests and clinical major symptoms for 3/7 patients.[12] In 4/7 patients, diagnosis was made according to their clinical presentations and laboratory results, with all three of the following criteria present: (1) Rapid onset of major groups of symptoms, (2) abnormal results in at least one of the laboratory tests, including EEG or CSF, (3) reasonable exclusion of other disorders.[12] The absence of antibodies does not exclude the possibility that a disorder is immune-mediated. At present, antibody testing is not accessible in TTMH and requires months to obtain results. The sensitivity of NMDA receptor antibody testing is higher in CSF than in serum, and a risk of false-negative or false-positive diagnoses exists if only serum is used, with up to 7% of patients demonstrating positive CSF titers with concurrent negative serum titers.[11]

CSF tests, EEGs, and brain images are typically non-diagnostic.[7] The majority of CSF tests show lymphocytic pleocytosis and oligoclonal bands, whereas EEGs may show slow activity without epileptic discharge.[5,8] However, the typical features present in CSF and brain images were not observed in the patients included in the present study.

The first-line treatments for anti-NMDA receptor encephalitis consist of IVIG, high-dose steroids, and plasma exchange.[1,10] In the present study, three patients received only antiepileptic drugs without immunosuppressant treatment; two of these patients showed substantial improvement and the remaining patient showed limited improvement. By contrast, of the four patients who received IVIG combined with a high-dose steroid, substantial improvement was observed in 2/4 patients and limited improvement was observed in 2/4 patients. Two of these four patients received immunosuppressant treatment, which led to the substantial recovery. These results suggest that IVIG treatment did not guarantee a favorable prognosis. Second-line treatments, such as rituximab or cyclophosphamide, or both, is usually required if there is no response.[1] In a case series of 10 patients with anti-NMDA receptor encephalitis, all patients were treated with immunotherapy in the acute period, and all patients showed good recovery.[13] At the end of follow-up in the present study, 4/7 (57.1%) patients had achieved substantial recovery following treatment with immunotherapy or antiepileptic drugs.

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抗 N- 甲基 -D- 天冬氨酸受體腦炎在台灣兒童的臨床表徵

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摘要

抗 N- 甲基 -D- 天冬氨酸受體腦炎是一種潛在致命的疾病，如果患者可早期得到診斷並接受適當治療，則很有可能恢復。由於此腦炎病人的臨床表徵多變且難以鑑別，大多數臨床醫師可能對此疾病不熟悉，本篇系統性的回顧分析將有助於一線的臨床醫師更了解此疾病。我們從 2007 年至 2015 年期間在童綜合醫院被診斷為抗 N- 甲基 -D- 天冬氨酸受體腦炎的入院患者進行了回顧性研究。分析其症狀、臨床特徵、實驗室數據、神經影像學檢查以及治療結果。本研究以腦脊液中的抗 N- 甲基 -D- 天冬氨酸受體抗體呈陽性反應配合臨床表現做確定診斷，以臨床表現和檢驗檢查結果做可能診斷。7 名抗 N- 甲基 -D- 天冬氨酸受體腦炎患者，年齡介於 4 歲至 13 歲之間（男性：女性：1：6）。85.7%（6/7）的患者表現出情緒，行為或性格改變；100%（7/7）患有口臉肌肉運動障礙；57.1%（4/7）有癲癇發作；7 名患者皆未發現任何腫瘤。57.1%（4/7）的患者接受靜脈注射免疫球蛋白治療。總體而言，57.1%（4/7）的患者達到良好的恢復。我們認為，抗 N- 甲基 -D- 天冬氨酸腦炎如能早期診斷，是可治療的疾病。本研究發現大部份患者有表現精神症狀，全部患者有口臉肌肉運動障礙，是此病的重要特徵。免疫球蛋白治療並不能保證患者有良好的預後，而有些患者的確在未使用免疫球蛋白治療下，也能自行恢復。

關鍵詞：抗 N- 甲基 -D- 天冬氨酸受體腦炎、自體免疫性腦炎、口臉肌肉運動障礙

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Case Report

An Incidental Pancreatic Neuroendocrine Tumor within a Pancreatic Pseudocyst treated with Laparoscopic Distal Pancreatectomy

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Abstract

Pancreatic cystic lesions are a type of lesion being increasingly detected, and account for approximately 5% of pancreatic neoplasms. Cystic tumors are often misdiagnosed as pancreatic pseudocysts (PPCs). A 27-year-old man visited our clinic due to abdominal pain and fever, and was diagnosed with a PPC. Due to persistent symptoms and multiple pseudocysts, a cystic neoplasm could not be ruled out. Laparoscopic spleen-preserving distal pancreatectomy was performed and pathology examination showed a 0.7-cm pancreatic neuroendocrine tumor (NET) within a pseudocystic formation. The post-operative course was uneventful and a seven-year follow-up did not show evidence of tumor recurrence. NETs are a group of heterogeneous tumors ranging from benign to high-grade malignant. A World Health Organization classification in 2010 categorized them into NET G1, NET G2, or neuroendocrine carcinoma G3. NET is rarely associated with acute or chronic pancreatitis. Therefore, diagnostic imaging is essential in young patients with pancreatitis of unknown cause. Surgical treatment provides the only possibility of cure in cases of NET G1.

Keywords: Neuroendocrine tumor, Pancreatic pseudocyst, Laparoscopic distal pancreatectomy

Introduction

Pancreatic cystic lesions are a type of lesion being increasingly detected, accounting for approximately 5% of pancreatic neoplasms.[1,2] Pancreatic cystic lesions include mucinous cystic neoplasms, intraductal mucinous neoplasms, serous cystadenoma, pancreatic ductal adenocarcinoma, or non-neoplastic cysts.[1] Cystic tumors are often misdiagnosed as pancreatic pseudocysts (PPCs).[2]

Pancreatic pseudocysts are complications of acute or chronic pancreatitis, pancreatic trauma, or pancreatic duct obstruction.[3] Their definition is based on fluid collection in the peripancreatic or intra-pancreatic tissues. They are surrounded by a well-defined wall and contain essentially no solid material.[3] Treatment of PPCs include conservation, percutaneous drainage, endoscopic transpapillary or transmural drainage, laparoscopic surgery, or open pseudocystoenterostomy.[3] The preoperative evaluation should exclude cystic neoplasms, which masquerade as PPCs.[3]

Pancreatic pseudocysts are sometimes very difficult to differentiate from other pancreatic cystic lesions. A mucinous cystadenocarcinoma of the pancreas might be initially misdiagnosed as PPC based on clinical evidence and radiological evidence including endoscopic ultrasound (EUS), computed tomography (CT), and magnetic resonance imaging findings.[4] Pancreatic cystic lesions are also very rarely found to coexist with pancreatic tumors. There has been only one previous report of the co-occurrence of a

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mucinous cystic neoplasm with a small pancreatic neuroendocrine tumor (NET),[5] and only one report of PPC co-occurrence with small adenocarcinoma.[6] Here, we report a very rare case of a small pancreatic NET within a PPC.

Case Report

A 27-year-old man visited our clinic with intermittent left upper abdominal pain radiating to the back, and low-grade fever for a few days prior. He was a heavy smoker and denied alcoholic use. He also denied any past history of gallstones or hyperlipidemia. Laboratory findings revealed elevated amylase and lipase. No specific positive physical exam was noted. EUS and CT scan were done and showed multiple PPCs (Fig. 1). After conservative treatment for four months, EUS and abdominal CT were repeated and showed multiple PPCs with progression. Due to the persistent symptoms and multiple pseudocysts, a cystic neoplasm could not be ruled out. Laparoscopic spleen-preserving distal pancreatectomy was performed and pathology evaluation showed a 0.7-cm NET (G1; Ki67, 2%) (Fig. 2) within a pseudocystic formation. The post-operative course was uneventful and a seven-year follow-up did not show evidence of tumor recurrence.

Discussion

NETs are a group of heterogeneous tumors ranging from benign to high-grade malignant. NETs can occur in almost every site of the body, especially in the lung, thymus, small intestine, rectum, stomach, cecum, colon, appendix, thymus, and pancreas.[7] The incidence of NETs is approximately 2.5 to 5 cases per 100000.[8] The most common locations of NETs are the lung and bronchus, followed by the small intestine and the rectum. The incidence of pancreatic NET (P-NET) is approximately 0.2–0.3 cases per 100000. NETs in the head, body, and tail of pancreas account for 44.9%, 12.3%, and 21.0% of the cases, respectively.[9]

According to the World Health Organization (WHO) classification in 2010, gastroenteropancreatic NETs (GEP-NETs) can be categorized into NET G1, NET G2, or neuroendocrine carcinoma (NEC) G3. NET G1 (Ki67 ≤ 2%) and G2 (Ki67 3%–20%) are tumor cells with well-differentiated morphology and Ki67 ≤ 20%. NECs have poorly differentiated histology with Ki67 > 20%.[7] NECs are also characterized by high-grade cytological atypia, extensive necrosis, apparent pleomorphism, and prominent mitotic activity.[7]

Treatment of P-NET includes surgical intervention, loco-regional radiofrequency ablation, laser ablation and cryotherapy, 90-Yttrium octreotide targeted radiotherapy, somatostatin analog-targeted systemic therapy, and systemic chemotherapy.[10,11] However, standard therapy is still lacking for P-NET, because of its rare, complex, heterogeneous nature, and poor understanding about it. Surgical treatment has been the primary and most important treatment for NETs, and is also the only possible treatment to achieve cure.[7] NETs are associated with delayed

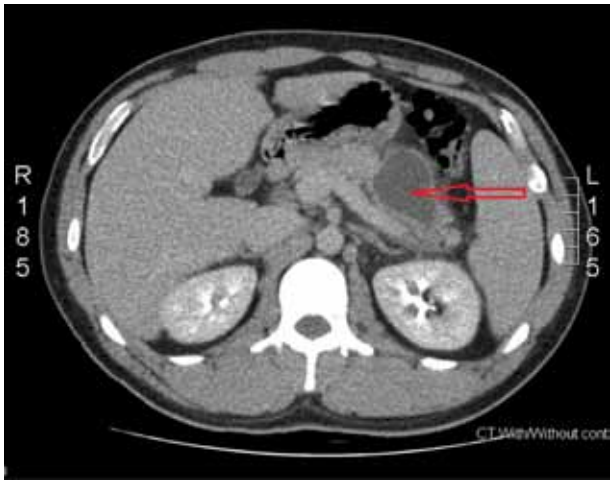

Fig. 1 Abdominal CT showed a pancreatic tail pseudocyst (arrow).

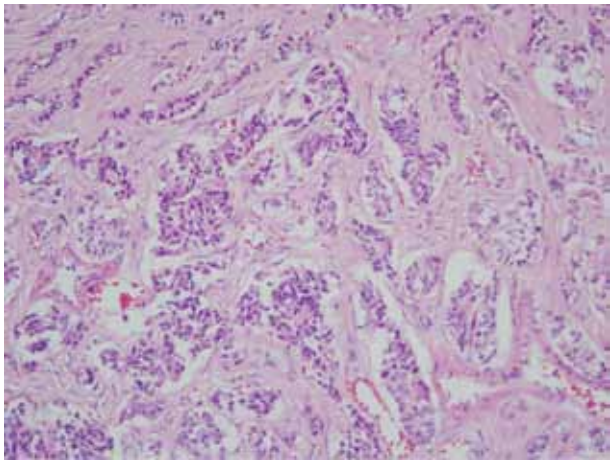

Fig. 2 Microscopic image of a neuroendocrine tumor of the pancreas. Tumor cells are arranged in small solid nests, surrounded by capillary vessels (Hematoxylin–Eosin staining).

clinical presentation, nonspecific symptoms and signs, and difficult-to-use biomarkers and imaging for early detection.[9] In the largest review of NET patients published to-date, which included 35825 cases, the statistically significant predictors were histological grade, disease stage, primary tumor site, race, sex, age, and time after diagnosis.[12] P-NET is very rare in Japan.[13] Shmomaura and colleagues[14] reviewed 40 cases of P-NET in Japan and found only three and one associated with acute and chronic pancreatitis, respectively. The present case of P-NET with chronic pancreatitis represents an extremely rare case. Detailed diagnostic imaging should be performed in young patients with pancreatitis of unknown cause. Our case was diagnosed as NET G1 according to the 2010 WHO classification, and surgical treatment was sufficient.

In conclusion, we present an extremely rare case of an incidental finding of pancreatic NET within a PPC. Detailed diagnostic imaging is essential for young patients with pancreatitis of unknown cause. Surgical treatment provides the only possibility of cure in cases of NET G1, and other methods of treatment require further study.

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腹腔鏡胰尾切除術治療意外發現於胰臟假性囊腫內的胰臟神經內分泌腫瘤

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摘要

胰臟囊性病變是近年來發生率持續在增加中，它佔約 5% 的胰腺腫瘤。囊性腫瘤常常被誤診為胰臟偽囊腫。一名二十七歲男子前往因為胰臟假性囊腫前來求診。由於持續的症狀和多發性偽囊腫而不能排除是囊性腫瘤。所以病人接受了腹腔鏡胰尾切除術。病理結果顯示 0.7cm 胰臟神經內分泌腫瘤發生於偽囊腫之內。術後恢復順利，追蹤七年內沒有發現腫瘤復發的證據。神經內分泌腫瘤是一群異質腫瘤，從良性到高度惡性的行為表現都有。2010 年世界衛生組織分類為神經內分泌腫瘤 G1 或神經內分泌腫瘤 G2 或神經內分泌癌 G3。神經內分泌腫瘤很少與急性或慢性胰臟炎有相關。因此，對於具有未知原因的胰臟炎的年輕患者，影像診斷上需要更加留意。同時外科治療是唯一提供神經內分泌腫瘤 G1 治癒的可能性。

關鍵詞：神經內分泌腫瘤、胰臟偽囊腫、腹腔鏡胰尾切除術

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Case Report

Synchronous Inflammatory Myofibroblastic Tumor of the Spleen with Acute Cholecystitis: A case report and literature review

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Abstract

Inflammatory myofibroblastic tumors (IMTs) are relatively uncommon neoplasms usually found in the lung. However, IMTs may also occur in extrapulmonary locations. We report a case of a 58-year-old man presenting with acute cholecystitis and an asymptomatic solid mass in the spleen. Laparoscopic cholecystectomy and splenectomy were performed and histologically proved the mass to be IMTs of the spleen. There was no tumor recurrent in the three-year follow-up. Laparoscopic splenectomy is safe and effective to treat splenic IMTs.

Keywords: Inflammatory myofibroblastic tumor, Spleen, Laparoscopic splenectomy

Introduction

Inflammatory myofibroblastic tumors (IMTs) are known histopathologically as an inflammatory pseudotumor, initially reported in the pulmonary system in 1939.[1] A number of terms have been applied to describe the lesion including, inflammatory pseudotumor, plasma cell granuloma, plasma cell pseudotumor, inflammatory fibroxanthoma, and most recently, inflammatory myofibroblastic tumor.[2] These tumors have been observed in many sites of the human body such as orbit, soft tissues, lymph nodes, heart, respiratory tract, gastrointestinal tract, liver and spleen.[3,4] IMTs are distinct entities and have specific immunohistochemical and molecular characteristics.[5] They are regarded as an intermediate malignant tumor with unknown etiology.[5] Splenic IMTs are often found incidentally, and diagnosis of splenic IMT in all reported cases was made after splenectomy, as the image of IMTs is similar to malignant tumors of the spleen. Only evaluations of histopathology and additional immunohistochemistry can reliably confirm IMT diagnosis.[4,5]

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Case Report

A 58-year-old man had a history of asymptomatic gallstones for years, and visited our hospital with chief complaints of persistent epigastric pain for a few days. Physical examination did not reveal organomegaly or lymphadenopathy but he was positive for Murphy's signs. Abdominal computed tomography showed gallstones and distal common bile duct (CBD) stones. In addition, a well-defined non-enhancing solid lesion measuring 4.2 × 3.5 × 3.2 cm was incidentally observed in the spleen (Fig. 1). A primary splenic tumor such as hamartoma or lymphoma was suspected. Laboratory findings determined white blood cells 7800/mm3, platelet count 279000/mm3, and C-reactive protein value of 4 mg/dL. Abnormal liver function tests were noted including: Glutamate

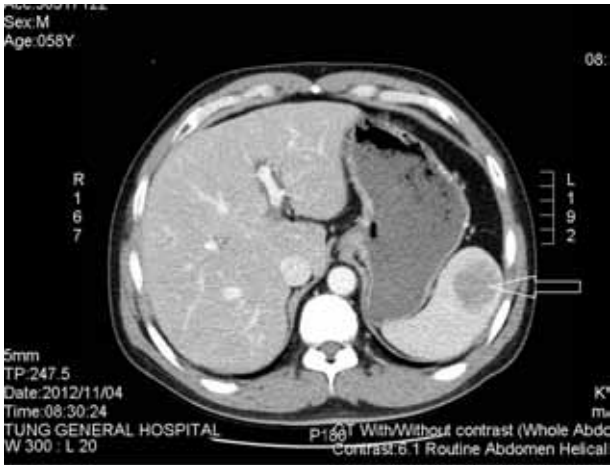

Fig. 1 Abdomen computed tomography (with contrast) showing a well-defined hypodense lesion in the spleen measuring 4.2 × 3.5 × 3.2 cm. (arrow head)

Fig 2ure Cut section showing a parenchymal pale, tan yellow-white, heterogeneous nodule measuring 4 × 3.4 cm. (arrow-head)

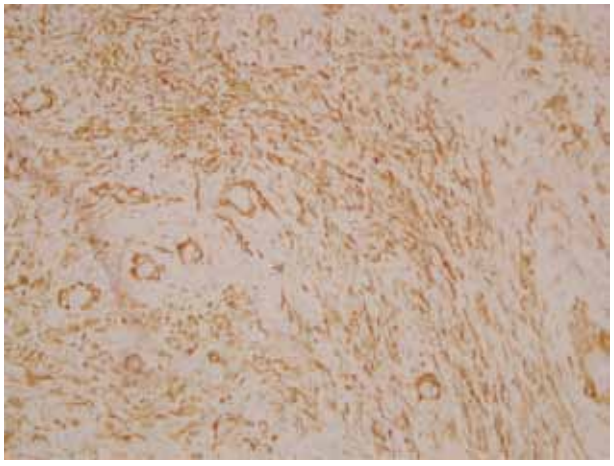

Fig. 3 Microscopic view showed spindle cells characteristically immunoreactive for SMA (IHC stain, ×400)

oxaloacetate transaminase (GOT) 394 IU/L; Glutamate pyruvate transaminase (GPT) 346 IU/L; Alkaline phosphatase (ALP) 155 IU/L; Direct bilirubin 1.3 mg/dL; Total bilirubin 2.4 mg/dL, and Gamma glutamyl transpeptidase (GGT) 628 IU/L. The patient was admitted, and then CBD stones were confirmed and removed using endoscopic retrograde cholangiopancreatography and endoscopic sphincterotomy with stone extraction. Then, the patient received a laparoscopic cholecystectomy and splenectomy five days later. The preoperative differential diagnosis included hamartoma, lymphoma, and single metastasis. The spleen specimens weighed 180 gm and measured 12 × 7 × 4 cm. The mass was roughly round and elastically hard. The cut surface of the mass was well-circumscribed, lobular, and pale tan yellow-white (Fig. 2). The size of the mass measured 4 × 3.4 × 3 cm in size and was located just beneath the spleen capsule. The lesion was 3 cm in distance to the hilar area, and the surgical margin of the spleen was free of tumorous tissue.

Microscopically, the lesion was characterized by a proliferation of spindle cells, small vessels, inflammatory cells, and presence of old hemorrhage. The inflammatory cells were gathered around blood vessels and forming germinal centers. Spindle cells were interspersed in the connective tissue. Immunohistochemistry showed that the tumor cells were positive for smooth muscle actin (Fig. 3), negative for ALK, and negative staining for S100 and CD117 (c-kit). A final diagnosis of inflammatory myofibroblastic tumor was made. Chronic cholecystitis with mucosa ulceration was noted at histological examination of the gall bladder. The postoperative course was uneventful and there was no evidence of tumor recurrence in 3 years of follow up without any adjuvant therapy. In addition, Epstein–Barr virus (EBV) antigen was detected on serum examination 3 months after discharge.

Discussion

A splenic inflammatory pseudotumor was first described by Cotelingam and Jaffe in 1984. They categorized the lesion as an inflammatory non-neoplastic reparative change.[6,7] IMTs are rare and specific lesions of unknown etiology. The most common location of IMTs is the lung, but almost any organ can be involved. The intra-abdominal sites of the disease are reported most frequently in the liver, followed

by stomach, bowel, spleen, mesentery, and extrahepatic bile duct.[8] IMTs occurring in the spleen are rather rare, comprising 3.2% of all primary splenic tumors.[8,9] There are some reports of synchronous diseases associated with IMT such as small cell carcinoma, Hodgkin's disease, colonic adenocarcinoma, cholecystitis, and adrenocortical adenoma.[10] Our patient also has synchronous chronic cholecystitis. Although the etiology and pathogenesis of IMTs are not entirely unknown, some etiologies have been proposed in their pathogenesis including infections, vascular causes, and autoimmune disorders.[10,11] Some cases have reportedly been caused by EBV infection.[11] In our case, EBV antigen was detected on serum examination. Recent work has reported chromosome rearrangement involving the ALK locus on chromosome 2p23 in both pulmonary and extrapulmonary IMT. The tumor nature of this neoplasm is different from other inflammatory pseudotumors and is regarded as an abnormal immunological reaction.[5]

Histologically, IMTs is comprised of proliferating spindle cells with variable inflammatory cells such as histiocytes, lymphocytes, and plasma cells. Immunohistochemically, the myofibroblastic spindle cells have tested positive for desmin, cytokeratin, vimentin, smooth muscle actin, muscle-specific actin, CD68 (KP-1), and CD30 (Ki-1).[5] In 2002, the World Health Organization classification of soft tissue tumors places IMTs in an intermediate category between benign and malignant, with a metastasis rate lower than 5%.[5,12] We agreed as a team not to perform ultrasonography-guided fine needle aspiration on the spleen, which presents with increased risk of uncontrollable bleeding and theoretical potential of tumor seeding in malignancy.[4]

Surgical treatments of both open and laparoscopic splenectomy are thought as the only available option to obtain reliable and definitive diagnosis and eventual cure.[4,5] The prognosis of IMTs is generally considered favorable with rare incidence of malignant transformation and remote metastasis following surgical treatment. Further radiation and chemotherapy are not recommended because of the generally benign course and intermittent potential of biology behavior.[4,5,8,13] However, reports of aggressive clinical courses of IMTs necessitate the need for close clinical follow-up.[4,5,8]

Laparoscopic splenectomy for IMTs results in less tissue trauma, reduced hospital stay and postoperative recovery period, and treatment of synchronous disease. In conclusion, laparoscopic surgery is a feasible and effective treatment for IMTs of the spleen in association with cholecystitis.

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脾臟發炎性纖維細胞腫瘤同時存在急性膽囊炎：病例報告和文獻回顧

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摘要

發炎症性肌成纖維細胞瘤是相對罕見的腫瘤。雖然肺是最熟知和最常見的部位，但發炎症性肌成纖維細胞瘤可能發生在肺外位置。我們報告一名 58 歲的男性患有急性膽囊炎和脾臟無症狀的實質腫塊。我們進行腹腔鏡膽囊切除術和脾切除術，病理報告證實為脾臟發炎症性肌成纖維細胞瘤。在三年的追蹤中沒有腫瘤復發的情況。所以腹腔鏡脾切除術對於脾的發炎症性肌成纖維細胞瘤是安全有效的。

關鍵詞：發炎性纖維細胞腫瘤、脾、腹腔鏡脾臟切除術

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Case Report

Mesenteric Paraganglioma: A Case Report and Review of the Literature

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Abstract

A symptomless mesenteric paraganglioma in a 69-year-old was an incidental finding during the performance of a routine physical examination. Paragangliomas rarely occur in a mesentery; only 18 cases have been reported to date. The case is presented with a brief summary of other cases, recent developments in the diagnosis, clinical characteristics, and pathology of this entity.

Keywords: Paraganglioma, Mesentery

Introduction

Paragangliomas are rare neuroendocrine tumors that arise from the extra-adrenal autonomic paraganglia. The annual incidence estimated as 1/100,000 population[2]. Most arise from parasympathetic or sympathetic tissue with 5%–10% of sporadic paragangliomas occurring at extra-adrenal sites[3]. Mesenteries are a rare location for paragangliomas as this case appears to be only the eighteenth cases reported to date[1].

Case report

A 69-year-old woman was admitted following the incidental discovery of an abdominal mass during a routine health-care examination. The patient was asymptomatic. Her medical history included hypertension that had been under control for more than 10 years. The physical examination on admission and the results of routine laboratory tests, including a liver function profile, were unremarkable. An abdominal ultrasound and computed tomography (CT) scan showed a 6.5 × 6.1 cm^2 hyperechoic hypervascular solid tumor in the right upper quadrant of abdomen abutting pancreatic head and second portion of duodenum (Fig. 1). A gastrointestinal tumor was suspected, and subsequent surgery revealed a round, well-lobulated 6.5 cm diameter mass in the mesentery near second portion of duodenum and adhered to pancreatic head (Fig. 1). The mass was completely resected without difficulty. No marked change of her preoperative blood pressure and postsurgical blood pressure. The patient's preoperative blood pressure ranged from 130/70 to 140/78 mm Hg with medication and did not change after surgery. Recurrence or metastasis was not found during 5-years of follow-up.

Pathological findings

The tumor was 7 × 5.5 × 5 cm on gross examination, lobulated, with a focal pseudocapsule, and firm and rubbery in consistency. The cut surface was brownish in color. Microscopically, the tumor was highly vascular and the tumor cells were patternless with focal sheet-like arrangements. Individual cells were spindle-shaped with clusters of round or polygonal cells with dense granular eosinophilic to amphophilic cytoplasm and centrally located oval nuclei with small inconspicuous nucleoli (Fig. 2). Neither tumor

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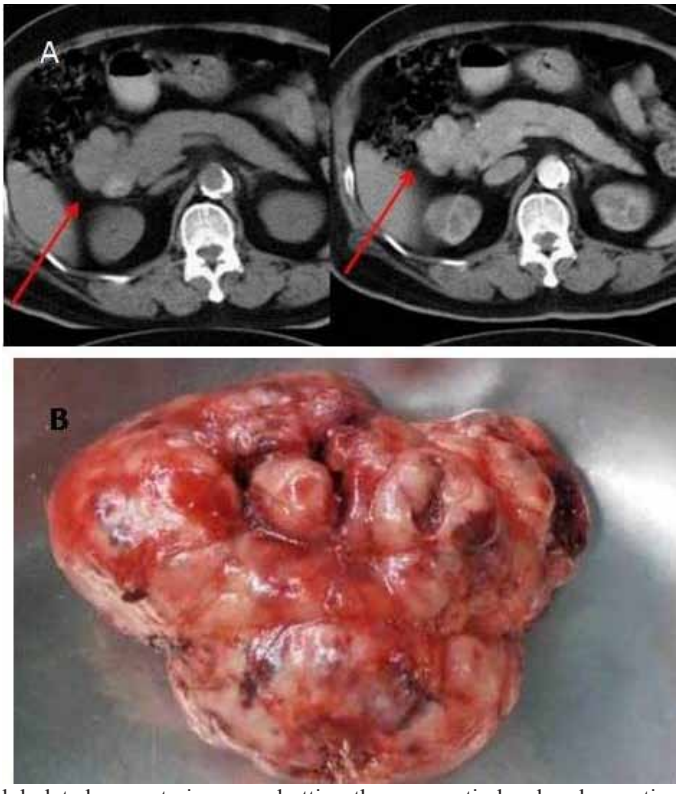

Fig. 1 (A) CT showing a well-lobulated mesenteric mass abutting the pancreatic head and a portion of the duodenum. (B) A lobulated, vascular mass with bleeding.

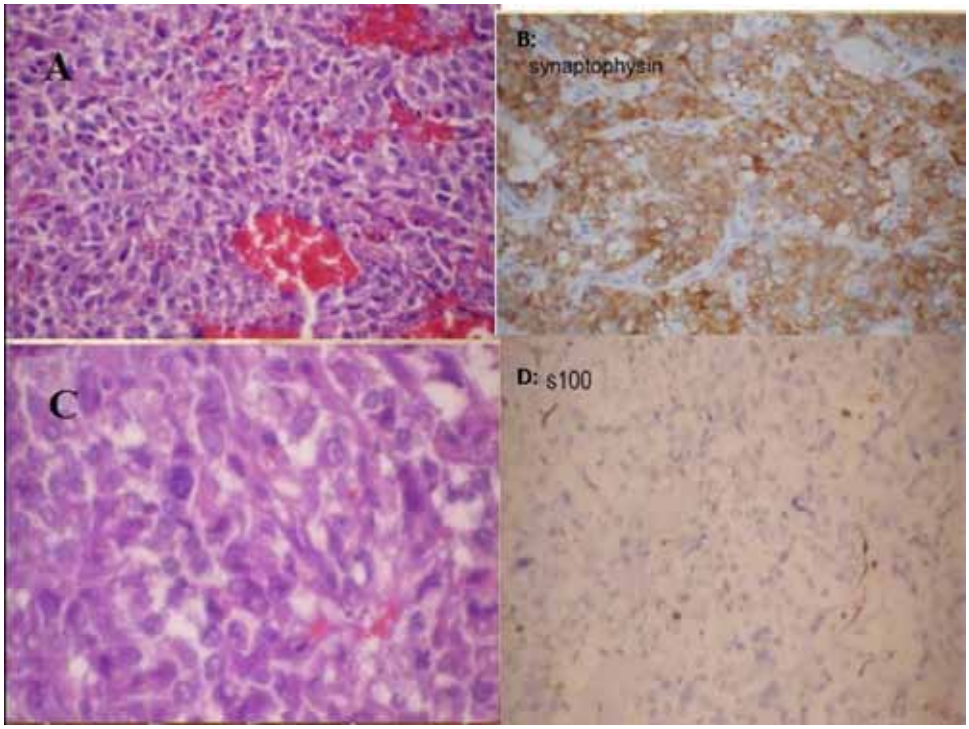

Fig. 2 (A) Spindle-shaped cells with patternless sheet-like arrangements with hypervascularity (100X, H&E). (B) Positive synaptophysin staining of tumor cells. (C) spindle-shaped cells with clusters of round to polygonal cells with finely granular eosinophilic to amphophilic cytoplasm and centrally located ovoid nuclei with small, inconspicuous nucleoli (400X, H&E). (D) S-100 focal-positive staining in sustenacular cells.

necrosis nor cellular pleomorphism were present, and the mitotic activity was very low (<1 figure per 10 high-power fields). Supporting sustentacular cells were absent in more than half the areas containing tumor cells, and marked spindling without a characteristic zellballen appearance or median to large supporting vascular channels were seen. The tumor cells were positive for chromogranin A and synaptophysin; S100 was positivity was focal, with a punctate or thin linear appearance (Fig. 2). Because of the tumor location and lack of the characteristic nest-like zellballen appearance, the differential diagnosis included gastrointestinal stromal tumor, melanoma, and metastatic poorly differentiated carcinoma. So, immunohistochemical stains of CD117, DOG-1, and smooth muscle actin were performed and negative results were found. Stain for CD34 was positive only in very focal vascular channels. Stains for keratin and HMB45 were also negative.

The pheochromocytoma of the adrenal gland scaled score (PASS) with Ki-67 was <3% with focal loss of sustenacular cells. Because of the rarity of mesenteric paraganglioma and presence of areas without sustentacular cells, the diagnosis of mesenteric paraganglioma was made after a pathological consultation. Recurrence or metastatic lesions have not been found after 5 years of follow-up.

Discussion

Approximately 85% of extra-adrenal paraganglioma arise from abdominal paraganglia adjacent to the aorta and often in the area corresponding to the organ of Zuckerkand[3]. The mesentery is a rare location. The reasons for development at unusual sites are not known, but presumed by Chetrit *et al.* to originate from mesenteric paraganglionic tissue[2]. The Glenner and Grimley classification, divides the paraganglionic system into four families, with mesenteric paraganglionic tissue included with the visceral-autonomic tissue near visceral organs and vessels[4]. It is distributed in mesenteric vessels in the duodenal wall, peripheral vessels, liver hilum, and bladder wall. The paraganglioma in our patient may have originated from mesenteric vessels adjacent to the duodenal wall.

In clinical practice the diagnosis of adult paraganglioma is a multidisciplinary effort that includes a patient history, biochemical testing, and multimodal imaging studies. Because of the rarity of mesenteric paraganglioma, and because most cases are nonfunctional, a preoperative diagnosis is difficult. Contrast CT usually shows a homogeneous or heterogeneous hyperenhancing soft-tissue mass and does not distinguish mesenteric paraganglioma from other mesenteric masses. Functional positron emission tomography may helpful in cases of functional paraganglioma because of increased fluorodeoxyglucose uptake by brown fat in the mesentery. It is believed that secretion of catecholamines by paraganglioma cells activates brown fat in unusual sites[5]. Preoperative diagnostic biopsy is helpful for abdominal masses, but if a paraganglioma is functional, performing a biopsy is associated with the risk of hypertension[6]. Fine needle aspiration is not recommended as it may result in significant hemorrhage and hypertensive crisis. Although it is rare, we recommend including paraganglioma in the preoperative differential diagnosis of solid hypervascular mesenteric tumors.

Pathological evaluation of paragangliomas is important because of its metastatic potential regardless of location. However, predicting metastatic behavior is difficult. In 2012, Thompson recommended use of the PASS score, which combines 12 histological features as a predictor[12]. Kulkarni *et al.* proposed that combining the PASS score, the number of S-100–positive sustenacular cells, a Ki-67 proliferative index of ≤3%, tumor location, and tumor size was better for risk stratification than the PASS scoring system alone[7]. Assadipour *et al.* reported a poor correlation of histological features and immunohistochemical staining with tumor behavior, and that *SDHB* gene mutation and tumor size were better for predicting tumor behavior[13]. Additional case evaluations are needed to better understand and predict metastatic potential.

A literature review (Table 1) found that 84% of the paragangliomas arising from mesentery were benign and 30% of cases presented with hypertension (30%). Some presented with an abdominal mass and nonspecific symptoms. Most were found in mesentery of small intestine, and 12 of 18 cases occurred in women. The mean age at diagnosis was 53 years and all cases were sporadic. No recurrences have been reported, but we recommend long-term postoperative follow-up.

Table 1. Clinical features of the 19 reported cases of mesenteric paraganglioma.

reference	age	sex	location	symptoms	sizes	hypertension	Preoprative diagnosis	metastasis	prognosis
Arean et al[3]	32	M	mesentery of small intestine	Nausea, vomiting diarrhea	10x7 cm^2	-	abdominal mass	-	no recurrence
Carmichael[3]	62	F	mesentery of small intestine	nausea, vomiting, back pain	3.2cm	present	abdominal mass	-	not documented
Tanaka[3]	29	F	descending colon	nausea, vomiting	10x9cm^2	-	retroperitoneal mass	Liver	32 months, no recurrence
Ishikura[3]	33	F	sigmoid colon	low abdominal pain, dysuria	15x15 cm^2	-	ovarian tumor	-	not documented
Onoue[3]	38	F	mesentery of small intestine	none	4.5x3.2 cm^2	-	mesenteric tumor	-	24 months, no recurrence
Jaffer[3]	76	M	mesentery of small intestine	abdominal mass, vomiting	8.5x8 cm^2	-	abdominal mass	-	not documented
Muzaffar[3]	76	F	mesentery of small intestine	abdominal mass	20x15 cm^2	-	abdominal mass	-	15 months, no recurrence
Ponsky[3]	35	F	mesentery of small intestine	abdominal mass, headach	5.5 cm	present	abdominal mass	-	24 months, no recurrence
Kudoh[3]	72	F	mesentery of small intestine (ileum)	abdominal mass, pain	10x9 cm^2	-	mesenteric tumor	-	12 months, no recurrence
Nobeyama[3]	53	M	mesentery of small intestine (ileum)	abdominal mass	15x10 cm^2	-	abdominal mass	-	not documented
Matsumoto[3]	77	F	mesentery of small intestine (near Bauhinn's valve)	abdominal mass	7x5.5 cm^2	-	mesenteric tumor	-	9 months, no recurrence
Chetrit[2]	55	M	mesentery of small intestine	abdominal mass	11.5x9.5 cm^2	-	mesenteric tumor	Lymph node	12 months, no recurrence
Fujuta[3]	78	F	mesentery of small intestine (near Bauhinn's valve)	none	3x1.5 cm^2	-	mesenteric tumor	-	8 months, no recurrence
Svajdler[8]	65	M	mesentery of small intestine (ileum)	none	10x8 cm^2	present	mesenteric tumor	-	3 months, no recurrence
Raghuveer M N[9]	23	M	mesentery of small intestine (jejunum)	mass in left hypochondriac region	10x10 cm^2	-	GIST of jejunum	-	15 months, no recurrence
P Nichkaode et al[10]	23	M	Mesentery of jejunum	abdominal pain, three masses	Up to 10x8 cm,	present	GIST with lymph node metastasis		not documented
Zeynep Ozkan et al[11]	59	F	Mesentery of terminal ileum	Infra- umbilical mass	6 cm	present	mesenteric tumor		not documented
Mohd Slim MA et al[1]	69	F	mesentery of small intestine	Abdominal mass	18x15x11.5 cm		Ovarian tumor	Lymphovascular invasion	not documented
presented case	69	F	mesentery of small intestine	none	6.5x6 cm^2	present	GIST	-	60 months, no recurrence

Conclusion

Paraganglioma arising from mesentery is very rare. Preoperative differential diagnosis is recommended of the risk of hypertension following needle biopsy or during surgery. Long-term follow-up is recommended because of the lack of reliable histological characteristics to predict malignant potential.

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腸系膜副神經節瘤：病例報告與文獻回顧

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摘要

我們提供一個發現於腸系膜的副神經節瘤案例。本例發生於一位 69 歲女性，臨床以腹部腫塊表示，無意間檢查發現的。就我們所知，發生在腸系膜的副神經節瘤十分罕見，文獻報告至今僅十八位。我們簡短摘要關於這類案例的臨床特徵，及最近在病理與診斷上的發展。

關鍵詞：腸系膜、副神經節瘤

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Case Report

Reverse Pseudohyperkalemia in a Patient with Acute Lymphoblastic Leukemia: A Case Report

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Abstract

Reverse pseudohyperkalemia is a rare condition, typically observed in patients with acute or chronic leukemia/lymphoma. Inappropriate potassium-lowering therapies in patients with reverse pseudohyperkalemia may result in hypokalemia and its associated deleterious effects. Here we describe a 61-year-old man who presented with severe hyperkalemia after the diagnosis of acute leukemia of ambiguous lineages, B/myeloid. Our case demonstrates that early recognition of reverse pseudohyperkalemia is challenging for clinicians and laboratory personnel and is a crucial aspect for treating such patients. With a high index of suspicion for this phenomenon, measuring potassium levels using a heparin-free collecting tube that is manually transported to the laboratory and immediately analyzing them can facilitate an early diagnosis and avoid mismanagement of such patients.

Keywords: Hyperkalemia, Pseudohyperkalemia, Reverse pseudohyperkalemia, Acute leukemia/lymphoma, Acute leukemia of ambiguous lineages, B/myeloid

Introduction

Hyperkalemia is a life-threatening electrolyte abnormality because of its associated cardiotoxic effects and the risk for fatal complications. Pseudohyperkalemia is an in vitro phenomenon wherein serum potassium levels are spuriously elevated compared with plasma levels. In contrast, reverse pseudohyperkalemia is defined as a normal serum potassium level but an elevated plasma level. Occurrence of both pseudohyperkalemia and reverse pseudohyperkalemia has been reported in patients with acute and chronic leukemia/lymphoma[1-7]. Distinguishing pseudohyperkalemia from true hyperkalemia is critical because unnecessary correction of spuriously elevated potassium levels in the former condition may lead to hypokalemia and its associated complications, whereas an urgent intervention to decrease plasma potassium levels is life-saving in patients with true hyperkalemia. Differentiation between pseudohyperkalemia and true hyperkalemia is challenging for both clinicians and laboratory staff. Here we describe a case of reverse pseudohyperkalemia in a patient with acute leukemia of ambiguous lineages, B/myeloid, to demonstrate the importance of prompt recognition of this phenomenon.

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Case Report

A 61-year-old man with an unremarkable past history presented to our emergency room with fever, jaundice, and lower leg edema since three days. Hematological investigations, including complete blood counts, were abnormal as follows: white blood cell (WBC) count, 480.3 × 10^3 (normal, 4–10 × 10^3) cells/μL; hemoglobin level, 4.0 (normal, 13–17) g/dL; hematocrit, 13.3% (normal, 41%–51%); and platelet count, 45 × 10^3 (140–520 × 10^3) cells/μL (Table 1). His

renal function was normal. Subsequent bone marrow biopsy and flow cytometry revealed bilineage tumor cells compatible with a diagnosis of acute leukemia of ambiguous lineages, B/myeloid; the blood result was predominantly that of acute precursor B lymphoblastic leukemia with a minor component of acute myeloid leukemia. His plasma potassium levels gradually increased from 6.7 meq/L (day 3) to 12.2 meq/L (day 7) during his hospital stay. Fluctuations of plasma potassium levels were noted during this period (Fig. 1). Because there were no obvious signs of hemolysis in any of the above blood specimens, the potassium level was analyzed using the same vial by two separate automated analyzers (UniCel DxC 800 Beckman Coulter, CA, US and Labospect 008 Hitachi, Tokyo, Japan); both showed similar extremely high potassium levels. However, the patient did not complain of fatigue, muscle cramps, or other cardiac or neurological symptoms associated with hyperkalemia. Furthermore, the patient's electrocardiogram (EKG) did not show typical changes associated with hyperkalemia. The patient was administered potassium-lowering therapy with insulin and oral sodium polystyrene sulfonate under the impression of tumor lysis-related hyperkalemia. Meanwhile, emergent hemodialysis was performed on day 3–6 of his hospital stay. Leukocytosis was observed during this period; WBC counts ranged from 393.3 × 10^3 to 232.4 × 10^3 cells/μL. However, he showed poor response to potassium-lowering therapies, and high plasma potassium levels persisted. Because the extremely high plasma potassium levels were inconsistent with the patient's clinical symptoms and EKG findings, reverse pseudohyperkalemia was suspected.

For the patient's acute leukemia, targeted therapy and chemotherapy with imatinib and vincristine were initiated on day 8. WBC counts decreased from 213.1 × 10^3 to 84.0 × 10^3 cells/μL from day 8 to day 10. To confirm our suspicion of reverse pseudohyperkalemia, additional series of blood samples were collected and manually processed (transported) from day 7 to day 12 (Fig 1). In addition, separate samples were simultaneously obtained in a lithium–heparin tube containing gel (Light green top BD Vacutainer® PST™ Π) and a yellow-top tube with a coagulant activator but without an anticoagulant (BD Vacutainer® SST™ Π Advance) on day 7. A discrepancy between plasma and serum potassium levels was observed (4.9 and 2.2 meq/L, respectively) (Table 2). The discrepancy resolved after day 8 along with a decrease in WBC counts owing to chemotherapy (Table 1). At the same time, the patient was found to have

Table 1. Laboratory Values of the patient on day 0 to day 7

Measurement items	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Reference Ranges
Sodium (meq/L)	126	130	132	132	–	129	–	–	137-150
Potassium (plasma) (meq/L)	4.9*	5.3*	4.9*	7.1*	11.3*	8.4*	8.3*	12.2*	3.5-5.3
Blood urea nitrogen (mg/dL)	12.0	9.0	8.0	10.0	–	13.0	–	16.0	7-22
Creatinine (mg/dL)	0.9	1.0	0.9	0.8	–	0.6	–	0.8	0.5-1.3
Glucose (mg/dL)	144	–	–	–	–	–	–	–	70-100
γ-GT (IU/L)	395	–	–	v	–	–	–	–	16-73
GOT (IU/L)	48	39	–	38	–	–	–	42	8-40
GPT (IU/L)	32	28	–	25	–	–	–	38	8-40
Total-bilirubin (mg/dL)	2.1	–	–	2.8	–	–	–	1.3	0.2-1.2
Albumin (g/dL)	3.0	–	–	–	–	–	–	3.1	3.4-5.3
White blood cell count (x10^3 cells/μL)	480.3	397.9	438.9	393.3	318.8	298.7	232.4	278.0	4-10
Hematocrit (%)	13.3	15.3	17.3	20.3	20.2	24.1	24.6	27.3	41-51 (Male)
Hemoglobin g/dL)	4.0	4.9	5.4	6.6	6.3	7.9	8.0	8.5	13-17 (Male)
Platelets (x 10^3 cells/μL)	45	37	64	55	66	53	43	34	140-520

#γ-GT, gamma-glutamyl transpeptidase; GOT, glutamyl oxaloacetic transaminase; GPT, glutamyl pyrubic transaminase
*The mark was the first plasma potassium data of the day.

Table 2. Comparison of simultaneous measurements obtained from various specimens.

	Day 7	Day 9	Day 10	Day 11	Day 12
Serum K (meq/L)	2.2 (653 U/L [a])	2.5	2.9	3.7	4.7
Plasma K (meq/L)	4.9 (840 U/L [a])	3.5	3.1	3.9	4.9

[a] Both serum and plasma lactate dehydrogenase levels were measured on day 7.
[b] All specimens were measured on UniCel® DxC 800 Beckman Coulter analyzer.

hypokalemia, and the serum potassium level was once lowered to 1.9 meq/L with an anticoagulant-free collecting tube compared with the plasma potassium level of 7 meq/L tested on the same day. Therefore, a potassium supplement was prescribed. In addition, a similar discrepancy with respect to plasma and serum levels of lactate dehydrogenase (LDH) (840 U/L and 653 U/L, respectively) (Table 2) was noted on day 7 using the same specimen as that used for potassium testing. His serum potassium level was maintained within the normal range during the rest of his hospital stay, whereas WBC counts continued to decrease with the progression of chemotherapy. Based on the above findings, a diagnosis of reverse pseudohyperkalemia was confirmed.

Discussion

Hyperkalemia is a critical electrolyte abnormality that requires prompt correction to avoid fatal complications. Because 98% of the body potassium is intracellular[8], even a mild extracellular release of potassium can lead to marked elevation of blood potassium levels. Clinically, several conditions such as reduced renal excretion, excessive potassium intake, or leakage of potassium from the intracellular space can lead to hyperkalemia[9].

In the laboratory, potassium levels may be measured in both serum and plasma samples. The difference in serum and plasma potassium levels is distinguished by the presence or absence of clotting factors. In our laboratory, a gel separator tube with a clot activator is used for serum samples and a tube with heparin as an anticoagulant is used for plasma samples. Serum potassium levels are 0.36 mmol/L higher than plasma potassium levels because platelets may release potassium during the clotting process[8].

Pseudohyperkalemia is defined as a marked elevation in serum potassium level compared with that in plasma potassium level in the absence of any symptoms of hyperkalemia or abnormal electrocardiogram[3,10]. As mentioned above, elevation in potassium levels may occur owing to the leakage of

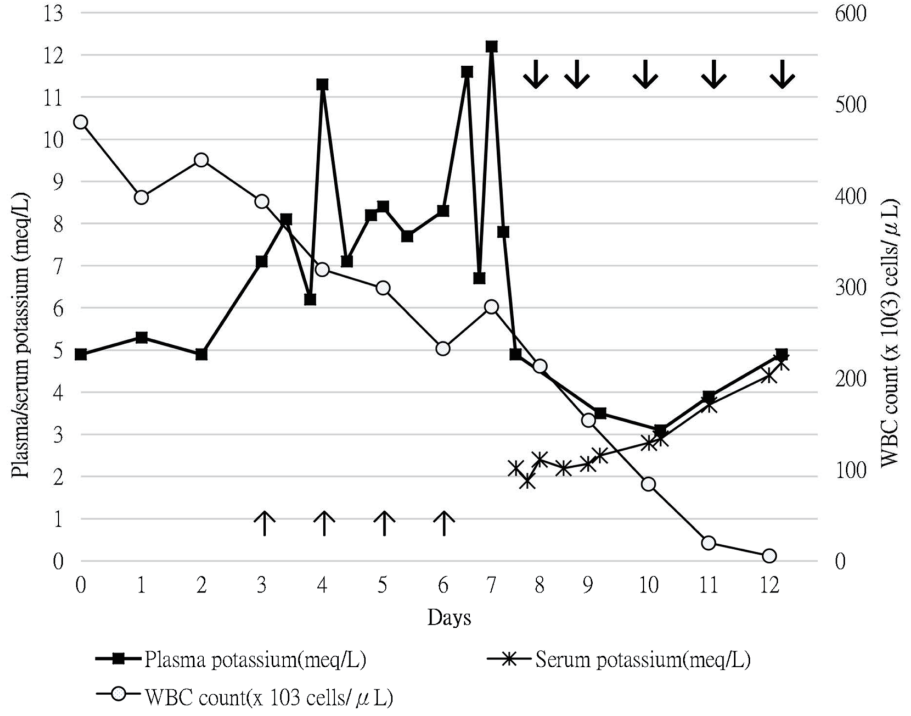

Fig. 1 Plasma potassium (■)and serum potassium (✻) levels and white blood cell counts (○) in a patient with acute leukemia of ambiguous lineages, B/myeloid. Potassium measurements in lithium heparin plasma samples via routine pneumatic tube transport showed apparent fluctuations of hyperkalemia during day 3 to day 7. In contrast, the manually transported serum samples after day 8 revealed hypokalemia and normokalemia. The case treated with dialysis and K lowering therapy during day 3 to day 7 (up arrowhead) and target therapy and chemotherapy initiated on day 8 (down arrowhead).

potassium from platelets during clot formation[11-13]. Furthermore, it is attributed to the release of potassium from cells during the process of specimen collection. Many pre-analytical factors such as mechanical factors (e.g., traumatic venipuncture and prolonged tourniquet use), contaminants, chemical factors (e.g., incomplete drying of ethanol), storage of specimen at inappropriate temperature, and delayed processing of blood samples can induce an artifactual abnormality[10,14].

Conversely, reverse pseudohyperkalemia refers to the condition when plasma potassium levels are spuriously elevated, whereas serum potassium levels are normal[4-7]. This phenomenon has been reported in blood samples of patients with acute or chronic leukemia or lymphoma. The precise underlying mechanism of this phenomenon is not well-characterized; however, several hypotheses have been proposed. One probable explanation is that the membrane of leukemic cells are fragile and particularly sensitive to heparin (an anticoagulant used in sampling tube for biochemical tests of plasma), which leads to significantly abnormal laboratory results (leakage of intracellular potassium)[5-7,15]. In a study by Meng et al., the degree of increase in plasma potassium levels was directly related to the amount of heparin[7]. In addition, mechanical stress such as that induced by pneumatic tube transportation system may cause cell lysis because of sudden acceleration[16,17]. Another postulated explanation is that in severe leukocytosis, higher consumption of metabolic fuels may lead to impaired Na^+/K^+ ATPase pump activity, possibly resulting in potassium release from the large number of WBCs[7,18,19]. An additional reason could be that a minority of leukemic cells undergo lysis in vitro and release potassium and cytoplasmic adenosine triphosphate in the plasma, which may increase cation permeability in vitro in leukemic cells of patients with chronic lymphocytic leukemia[6,20,21]. For a more accurate measurement of potassium levels in the setting of reverse pseudohyperkalemia, several studies have recommended the use of arterial whole blood with a blood gas analyzer because it precludes the delay in processing and centrifugation, causes the least degree of mechanical stress, and does not require the use of a tourniquet or pneumatic tube transport[2,22,23]. A serum specimen processed via manual transport yields similar results for potassium levels as those obtained with a blood gas analyzer and could be a viable alternative to the use of lithium heparin plasma with pneumatic tube transport[23]. Manual transportation of a specimen may be preferred to minimize the effect of mechanical stress that may cause disruption of fragile leukemic cells during sample delivery.

In our case, potassium and LDH levels measured using lithium–heparin plasma were both higher than those measured in serum obtained from non-hemolyzed specimens. The results suggest that our routine method for measuring plasma potassium levels is inappropriate for such patients. Based on our experience with our case, the measurement of serum potassium level is suggested when reverse pseudohyperkalemia is strongly suspected. Furthermore, to facilitate early recognition of reverse pseudohyperkalemia, an alarm mechanism has been set up in our laboratory information system for patients with hyperkalemia (>6 meq/L) and extreme leukocytosis (>100 × 10^3 cells/μL) to make clinicians and laboratory staff aware regarding the possibility of this rare condition.

In conclusion, our experience with this patient demonstrates that reverse pseudohyperkalemia should be considered in the differential diagnosis of hyperkalemia in patients with hematological neoplasms who present with severe leukocytosis, particularly those presenting with the absence of compatible clinical symptoms and EKG changes. Once reverse pseudohyperkalemia is suspected, repeat measurement of the potassium level with a heparin-free collecting tube via manual transportation of specimens and immediate analysis can help obtain a correct measurement. An early recognition of this uncommon phenomenon is crucial for appropriate treating these patients

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一位急性淋巴性白血病人的反向假性高血鉀現象

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摘要

反向假性高血鉀是一種少見的現象，通常發生於急性或慢性白血病 / 淋巴瘤之患者。臨床上對高血鉀症的治療可能導致具有反向假性高血鉀現象的患者轉變為低鉀血症及造成傷害。我們報導一名 61 歲診斷為 acute leukemia of ambiguous lineages, B/myeloid 的男性出現反向假性高血鉀之案例，本案例顯示臨床醫師和醫檢師儘早識別出反向假性高血鉀現象對患者的治療是非常重要的，當高度懷疑病患出現反向假性高血鉀現象時，以不含肝素的採血管採集血液，以人工運送方式送至檢驗室並立即檢測血鉀濃度，可有助於早期診斷及避免錯誤醫療處置。

關鍵詞：高血鉀、假性高血鉀、反向假性高血鉀、急性白血病

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Case Report

Acute Dystonia after the Administration of Aripiprazole at a Subtherapeutic Dose in A Neuroleptic-Naive Psychotic Patient: A case Report and Literature Review

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Abstract

Background and purpose: To report a case of acute dystonia developed after treatment with aripiprazole at a subtherapeutic dose

Methods: A 34-year-old neuroleptic-naive man with new-onset psychosis developed acute dystonia after undergoing treatment with aripiprazole at a subtherapeutic dose (7.5 mg/day).

Results: In most cases, acute dystonia developed with 10–30 mg/day of aripiprazole. Herein, we report the first case of acute dystonia caused by aripiprazole administration at a subtherapeutic dose, i.e., 7.5 mg/day; the patient was a 34-year-old neuroleptic-naive man with new-onset psychosis. Further, we provide an updated literature review on aripiprazole-associated acute dystonia.

Discussion: This case highlights the need for the awareness of clinicians about acute dystonic reaction in patients treated with aripiprazole at a subtherapeutic dose, although this dose is known for its low propensity to develop extrapyramidal syndrome.

Keywords: Antipsychotics, Aripiprazole, Acute dystonia

Introduction

Aripiprazole is known for its low propensity to cause extrapyramidal syndrome (EPS) because of its partial agonism on dopamine activity in the nigrostriatal tract. However, accumulating evidence has revealed that aripiprazole not only is associated with minimal EPS but may also cause serious, life-threatening adverse events such as acute dystonia[1,2]. In most cases, acute dystonia developed following treatment with therapeutic doses of aripiprazole at 10–30 mg/day[3-5]. Ittasakul et al[6] reported acute dystonia (torticollis) in a 21-year-old schizophrenic woman after treatment with low-dose aripiprazole (5 mg/day), but previous use of typical antipsychotics may also contribute to dystonia, weakening the causal relationship between aripiprazole and acute dystonia. Herein, we report a case of acute dystonia that developed after treatment with aripiprazole at a subtherapeutic dose (7.5 mg/day) in a 34-year-old neuroleptic-naive man with new-onset psychosis. We also provide an updated literature review on aripiprazole-associated acute dystonia.

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The case

A 34-year-old man presented to our psychiatric outpatient department with paranoid delusion and auditory hallucination (AH). He claimed that he had developed delusions of being persecuted, referred, and monitored over the past 3 months. Further, his mother confirmed his suspiciousness, excessive

anxiety when going outdoors, and preoccupation with thoughts of being set up by unfamiliar strangers. In addition, he experienced intense voice-commanding and voice-commenting AHs, which were mostly from his dead grandmother. He had no history of developmental abnormality, substance use disorder, and head injury. Although he had a history of reflux esophagitis and mitral valve prolapse, he did not use associated therapeutic agents for at least 1 year. Accordingly, the diagnosis of paranoid schizophrenia was favored, and he was administered aripiprazole at a dose of 7.5 mg/day on his first visit. In addition, 10 mg prophylactic propranolol was administered twice daily for the prevention of the likely development of akathisia and 10 mg zolpidem at bedtime for insomnia. He also complained of only mild dizziness at the first follow-up on day 7, but he apparently developed EPS by the subsequent follow-up on day 14. Further, he developed muscle stiffness in his limbs and jaw, speech interruptions, dysarthria, and mild dysphagia. In addition, he was unable to stand upright and bend toward the right side. Examination revealed dystonic scoliosis of the thoracic spine with convexity to the left. He also developed a severe sensation of inner restlessness and kept stepping and pacing in the office. Apart from these findings, fever as well as hematological and biochemical abnormalities were not observed. Based on the clinical and laboratory examinations, he was diagnosed with acute dystonia and akathisia. The dystonic reaction subsided immediately after the administration of a prompt intramuscular injection of 5 mg biperiden. Aripiprazole was discontinued, and 5 mg/day olanzapine and 4 mg/day trihexyphenidyl were prescribed instead. After 1 week, EPS and akathisia disappeared. Although minimal improvement of psychotic symptoms was achieved, we decided to continue the administration of 5 mg/day olanzapine to prevent another possible episode of dystonia. To date, he has been administered 5 mg/day olanzapine for >6 months without the development of dystonia.

Discussion

Aripiprazole is a dopamine partial agonist and serotonin 2A receptor (5-HT_{2A}) antagonist. It has a favorable adverse effect profile and very low reported incidence of EPS, with akathisia being the most common adverse effect. However, an increasing number of cases of aripiprazole-associated acute dystonia have been reported over the past decade (Table 1). The exact mechanism underlying antipsychotic-induced acute dystonia remains unclear, but it is possibly attributable to a higher ratio of dopamine–acetylcholine antagonism in the basal ganglia. The property of high affinity at dopamine D_2 receptor and low affinity at muscarinic receptor may subject patients to a greater risk of dystonia[7]. Approximately 92% cases of acute dystonia (11 of 12 cases) have developed following the administration of aripiprazole at recommended therapeutic doses between 10 and 30 mg/day. However, most patients developed comorbidities with the concomitant use of psychiatric agents (valproate[8], lithium[9] and selective serotonin reuptake inhibitors[2,5,10]) or substance use (cocaine[11] and amphetamine[12]). These agents may increase the risk of acute dystonia directly or through drug–drug interactions. Although Ittasakul et al[6] reported acute dystonia after treatment with aripiprazole at a lower dose of 5 mg/day (7.5 mg/day in our patient) in a 21-year-old schizophrenic woman, typical antipsychotics (perphenazine and trifluoperazine) had been previously prescribed for several days. Administration of relatively high-potency antipsychotics is more likely to cause acute dystonia, and dystonia may occur while the plasma concentration of neuroleptic agents is reducing after an abrupt cessation of previous agents. Thus, we believe that this is the first case report on acute dystonia caused by treatment with aripiprazole at a subtherapeutic dose of 7.5 mg/day in a neuroleptic-naive patient (Naranjo score: 7). Several risk factors for acute dystonia in this patient, including young age, male sex, and Asian race, could explain the occurrence of acute dystonia with aripiprazole administration at a subtherapeutic dose, whereas being neuroleptic naive clearly implicates aripiprazole as the causative factor. However, aripiprazole is metabolized mostly via cytochrome P450 2D6 (CYP2D6) and cytochrome P450 3A4 (CYP3A4). Thus, the likely explanation for acute dystonia in this case could be an increased plasma aripiprazole level due to the inhibition of CYP 2D6 by propranolol or of CYP 3A4 by zolpidem, for which both are substrates. Another noteworthy finding is that acute dystonia developed 2 weeks after the initiation of aripiprazole-based treatment in our patient, whereas most of the other patients developed the reaction within 3 days. The half-life of aripipazole is 75 h, and it is reasonable to use aripipazole for 5 half-lives (approximately

Table 1. Literature review of case reports of aripiprazole-associated acute dystonia

	Age	Sex	Diagnosis and comorbidity	Dose of aripiprazole at onset of dystonia	Clinical features of dystonia	Concomitant medication or substance use	Time to onset of dystonia following aripiprazole
Fountoulakis KN (2006)[3]	18	M	Tourette's disorder	10 mg/day	Facial muscle spasm, Oculogyric crisis, torticollis	None	3 days
Desarkar P (2006)[8]	18	M	Bipolar disorder	15 mg/day	Torticollis	Valproate 1000 mg/day	3 days
Singh MK (2007)[4]	10	M	Bipolar disorder	10 mg/day	Torticollis	Guanfacine 0.5 mg/day	3 days
Sanghadia M (2007)[10]	19	F	Schizophrenia Obsessive compulsive disorder	15 mg/day	Lower jaw protruding to the right, Torticollis	Sertraline 200 mg/day	3 days
Henderson JB(2007)[11]	58	M	Bipolar II disorder Cocaine dependence History of Attention-deficit/ hyperactivity disorder (ADHD) and Panic disorder with agoraphobia	10 mg/day	Jaw muscle contraction	Cocaine Hydrochlorothiazide Lisinopril Methocarbamol Misoprostol Piroxicam simvastatin	2 hours
Shen YC (2008)[12]	25	M	Amphetamine-induced psychosis	10 mg/day	Torticollis	Amphetamine	3 days
McLaren JL (2010)[9]	11	M	ADHD Bipolar disorder	30 mg/day	Spasmodic muscular contractions of jaw Forceful jaw closure	*Extended-release OROS methylphenidate 108 mg/day Lithium 900 mg/day	2 years *33 hours after last dose of methylphenidate
Solomon S (2010)[2]	23	F	Depression	10 mg/day	Left-sided temporomandibular dislocation	Escitalopram 10 mg/day	20 hours
Ittasakul P (2012)[6]	21	M	Schizophrenia	5 mg/day	Torticollis	None	3 days
Goga JK (2012)[1]	16	F	Bipolar I or II disorder Anxiety disorder Posttraumatic stress disorder History of ADHD	10 mg/day	Dyspnea, dysphonia, tongue and throat tightening (laryngeal dystonia)	Topiramate 50 mg/day Naproxen 750 mg/day	3 days
Saddichha S (2012)[5]	33	F	Schizoaffective disorder	30 mg/day	Acute neck dystonia	Fluoxetine 20 mg/day	1 week
Chen MH (2013)[10]	32	F	Bipolar I disorder	20 mg/day	Lordortic position	Not mentioned	1 week

*Extended-release OROS methylphenidate was discontinued when acute dystonia developed.
M= male, F= female

2 weeks) to reach a steady plasma level. This may explain the phenomenon of delayed onset of dystonia. Despite the delayed dystonia reaction in our patient, the aforementioned clinical presentation does not satisfy the operational criteria for the diagnosis of tardive dystonia characterized more by the chronic nature of dystonia than by the duration of antipsychotic exposure. Differential neuroleptic malignant syndrome should be considered, but it was not likely in our patient because fever did not develop during the aripiprazole treatment course.

This case highlights the need for the awareness of clinicians about an acute dystonic reaction during the treatment of neuroleptic-naive psychotic patients with aripiprazole, which is known for its low propensity to develop EPS, even at a subtherapeutic dose.

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一位從未接受抗精神病劑治療的精神病人服用低於建議治療劑量的大塚安立復後產生急性肌張力不全：個案報告及文獻回顧

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摘要

背景及目的：報告一案例在使用低於建議治療劑量下之抗精神病劑大塚安立復（aripiprazole）後發生急性肌張力不全（acute dystonia）。

方法：一位 34 歲第一次精神病發病之男性，使用低於建議治療劑量下之抗精神病劑大塚安立復 7.5mg/d 後發生急性肌張力不全。

結果：大部分急性肌張力不全的個案都是發生在大塚安立復的建議治療劑量下 10-30 mg/day。此處，我們報告了第一位在使用低於建議治療劑量下之抗精神病劑大塚安立復 7.5 mg/day 後發生急性肌張力不全的 34 歲男性且第一次發病及從未使用過抗精神病劑。我們同時提供了和大塚安立復相關之最新急性肌張力不全的文獻回顧。

討論：本案例提醒臨床工作者要特別注意，即使大塚安立復發生錐體外症候群的機會較低，但在建議治療劑量以下仍可能發生。

關鍵詞：抗精神病劑、大塚安立復、急性肌張力不全

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Case Report

Cervical Spinal Epidural Abscess: A Case Report

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Abstract

A cervical spinal epidural abscess (CSEA) in the neck is often life-threatening. However, this disease is difficult to diagnose in the early stage. Patients with symptoms of a CSEA, such as fever, back/neck pain, and neurological deficits, need to be further assessed using magnetic resonance imaging (MRI) to confirm the diagnosis. Currently, a CSEA is widely treated with surgical decompression, followed by antibiotic treatment. Here, we report a case of a CSEA associated with neurological issues, which was successfully treated with surgery and showed neurological function recovery in approximately two weeks after surgery.

Keywords: Cervical epidural abscess, Neurologic deficits

Introduction

A cervical spinal epidural abscess (CSEA) is an uncommon disease with diverse clinical symptoms and signs. Clinically, this disease is difficult to diagnose, especially in the early stage[1]. When a patient experiences fever, back pain, and progressive neurological deficits, such as quadriparesis, sphincter dysfunction, and a detectable sensory level, a CSEA should be considered as the underlying disease[1,2]. Here, we report a case of a CSEA that was successfully treated with surgery and review the literature.

Case Report

A 59-year-old man was admitted to our hospital for neurological issues. He had histories of hypertension, diabetes mellitus (DM), and alcoholism, and he had been treated at a local clinic. He experienced ischemic stroke with left (Lt) hemiparesis in May 2011 and complained of dull pain in the posterior neck for several weeks. He could not get up after awakening on the morning of the admission day. In addition, low-grade fever (temperature between 37°C and 38°C) was noted in the previous few days. After admission, relevant laboratory data showed no leukocytosis (7,700/μL) on complete blood count and a C-reactive protein level of 5.0 mg/dL. Neurologically, the patient showed quadriparesis, with muscle power values of 3/5 for both arms, 4/5 for the Lt leg, and 2/5 for the right (Rt) leg. Brain MRI showed old infarcts at the Rt occipital and pons areas. On the second day of admission, quadriparesis worsened, with muscle power values of 2/5 for both arms and 0/5 for both legs. MRI of the C-spine showed an epidural abscess with spinal cord compression and edematous change of the spinal cord at the C4–7 levels (Fig. 1). A neurosurgeon was consulted immediately, and surgery was performed several hours later. About 10 days after the surgery, the muscle power values improved to 5/5 for the arms and 4/5 for the legs. The microorganism identified on culture of the abscess fluid was *Streptococcus agalactiae*.

Discussion

A CSEA is a rare medical emergency, accounting for 0.2-1.2 cases per 10,000 hospital admissions per year[3], and if left untreated, it can result in

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catastrophic and irreversible neurological damage. In the developed world, the organisms most frequently associated with a CSEA are *Staphylococcus aureus* (57%-93% of cases), *Streptococci* (18%-69%), including *S. agalactiae*, *S. anginosus*, and *S. constellatus*, and a variety of gram-negative bacilli (13%) [4-6]. The most common risk factor is DM, followed by trauma, acupuncture, intravenous drug abuse, and alcoholism[7,8]. Fever, pain in the neck, chest, or back, and progressive neurological deficits are considered as the symptom triad of a spinal epidural abscess[1-3,9]. However, a spinal epidural abscess causing all these typical symptoms and signs is very rare, and the disease is especially difficult to diagnose in the early stage because of the diversity of the clinical symptoms and signs[1,10]. The correct diagnosis may be suspected in only 40% of patients at the time of presentation[11], because pathologies associated with back pain, fever, and spinal tenderness are more common in other diseases than in an epidural abscess. Current reviews have concluded that early surgical decompression and prolonged (6-12 weeks) antibiotic therapy (intravenous followed by oral) are the primary treatment approaches[12,13]. Among CSEA cases, antibiotics should be used first and/or concomitantly with open surgery in severe forms with spinal cord compression and antibiotics should be used as first-line treatment in milder forms without significant spinal cord compression.

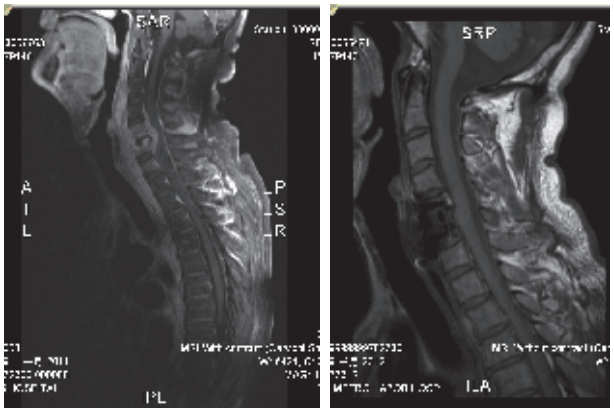

Fig. 1 Magnetic resonance imaging of the lesion of the cervical spinal epidural abscess at the C4–7 levels before (left) and after surgery (right)

Conclusion

The findings of the present case suggest that it is crucial to consult with a neurosurgeon immediately after the diagnosis of a CSEA associated with neurological deficits and spinal cord compression to achieve good recovery.

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頸脊髓硬膜外膿腫：病例報告

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摘要

頸脊髓硬膜外膿腫常常會危及生命。然而，欲在早期診斷這種疾病並不容易。因為頸脊髓硬膜外膿腫的症狀包括如發燒，背部 / 頸部疼痛以及神經缺陷等等，容易和其他疾病混淆，所以確切診斷還需以磁振造影影像的檢查來確定。目前，頸脊髓硬膜外膿腫的治療主要是靠手術減壓再加上抗生素治療。我們在這裡報告了一個頸脊硬膜外膿腫的病例，病患經手術成功的治療並在兩週後恢復神經功能。

關鍵詞：頸脊髓硬膜外膿腫、神經缺陷

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Image

Correlation of Scrotal Ultrasound Findings with Pathological Features in Intratesticular Epidermoid Cyst, A Prepubertal-Type Teratoma

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Abstract

Intratesticular epidermoid cysts are rare benign tumors of the testes. Based on the World Health Organization classification of the tumors of the urinary system and male genital organs (2016), testicular epidermoid cysts are classified as prepubertal-type teratomas. We herein present the case of a 30-year-old male suffering from a left testicular mass for three months. Physical examination confirmed a 2-cm, hard, nontender testicular mass in the left scrotum. Ultrasonography revealed a 2.1 × 2.1-cm solid mass without hypervascularity in the lower testicular pole. Serum α-fetoprotein and human chorionic gonadotropin levels were normal. Orchidectomy was performed based on the preoperative diagnosis of benign left testicular tumor, which was pathologically confirmed as intratesticular epidermoid cyst, a prepubertal-type teratoma. We also review the literature and discuss the correlation of scrotal ultrasound findings with pathological features.

Keywords: Intratesticular epidermoid cyst, Prepubertal-type teratoma

Image Page

A 30-year-old male presented with a three-month history of a left testicular mass noted by self-examination. Physical examination confirmed a 2-cm, hard, nontender testicular mass in the left scrotum. Sonographic findings demonstrated a 2.1 × 2.1-cm solid mass without hypervascularity in the lower testicular pole. Serum α-fetoprotein and human chorionic gonadotropin levels were normal. The preoperative diagnosis was benign left testicular tumor, and orchidectomy was performed.

Gross pathological examination revealed an intratesticular tumor comprising a well demarcated and encapsulated soft white-yellow mass measuring 2.1 × 2.0 cm. Bisection of the lesion revealed a solid mass with concentric rings, resembling an onion peel, comprising white-yellow amorphous and paste-like material within the lesion. The lesion also showed a target/bull's eye feature in the center (Fig 2). Microscopically, keratinizing squamous epithelium was observed to surround the keratin-filled and laminated cyst with keratinized debris in the center (Fig 3). The lesion was surrounded by normal seminiferous tubules and a delicate fibrous wall (Fig 4). The cyst lumen contained keratinized debris in the center (Fig 5). There were no skin appendages or cytologic atypia. The definitive diagnosis was intratesticular epidermoid cyst.

The pathologic diagnosis of epidermoid cysts is based on the criteria proposed by Price that include the following: (a) the lesion must be cystic and intraparenchymal; (b) the lumen should contain keratin with no teratomatous elements or dermal adnexal structures such as sebaceous glands or hair follicles;

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(c) the cyst walls should comprise fibrous tissue with a complete or incomplete inner lining of squamous epithelium; and (d) the remaining testicular parenchyma may be atrophic but must not contain teratomatous elements, *in situ* germinal tumors, or scarring[1].

Epidermoid cysts of the testes, first described in 1942 by Dockerty and Priestley[2], are uncommon and account for 1.5%–2% of all testicular tumors. The majority of patients are in the second to fourth decades of life, although patient age ranges from 3 to 77 years. The histogenesis of epidermoid cysts is not completely understood, and several theories were proposed to explain their embryologic origin. Metaplasia of the seminiferous epithelium or rete testis has been suggested; however, most studies suggest that these tumors are a result of the monodermal development of a teratoma without evidence of malignancy[1]. Based on the World Health Organization classification of the tumors of the urinary system and male genital organs (2016), the testicular epidermoid cysts are classified as prepubertal-type teratomas (Fig 6), germ cell tumors that are usually found in the prepubertal testes and composed of elements resembling somatic tissue derived from one or more germ cell layers. Prepubertal-type teratoma shows no association with germ cell neoplasia *in situ*, dysgenetic parenchymal changes, scarring, or chromosome 12p amplification. In post-pubertal patients, these are sometimes called benign post-pubertal teratomas; however, the designation of prepubertal-type

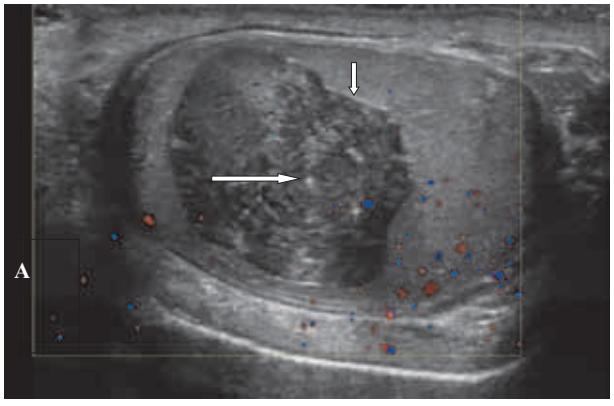

Fig. 1 Scrotal ultrasonography showing a sharply defined mass with a hyperechoic rim and an onion-peel appearance (short arrow). A "target/bull's eye" pattern can also be observed in the center of the mass (long arrow). (Retrospective review).

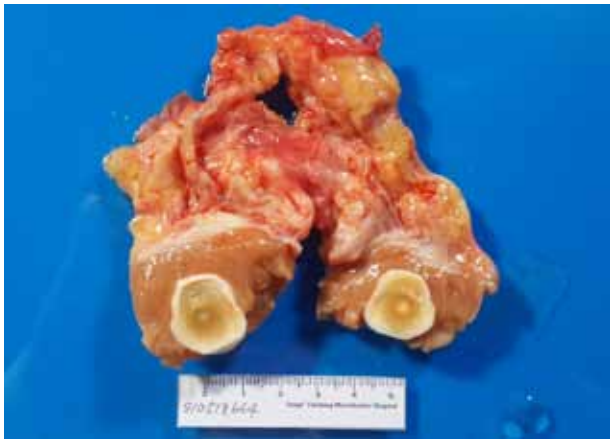

Fig. 2 A 2.1 × 2.0-cm, white-yellow intratesticular tumor exhibiting concentric ring (onion peel) and target/bulls-eye features in the center (arrow).

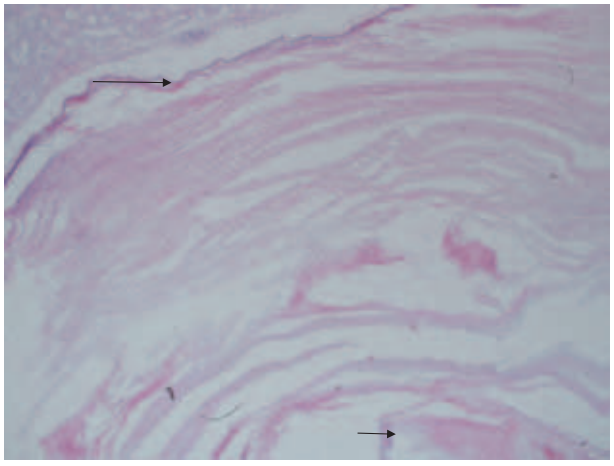

Fig. 3 Keratinizing squamous epithelium is observed to surround the keratin-filled and laminated cyst (long arrow) with keratinized debris in the center (short arrow) (hematoxylin/eosin stain, 20×).

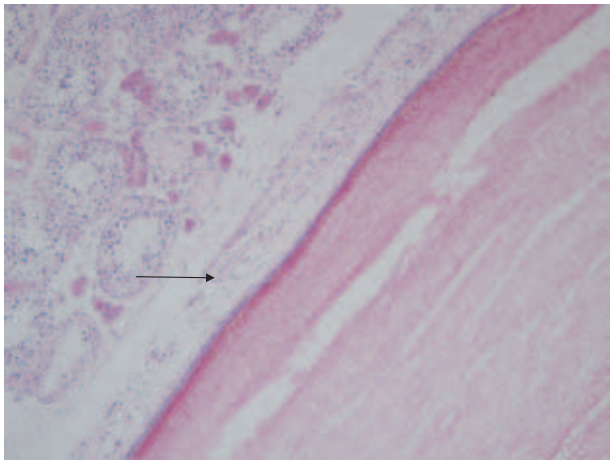

Fig. 4 Normal semiferous tubules are in the left, and keratinizing squamous epithelium is surrounding the keratin-filled and laminated cyst in the right. The delicate fibrous wall of the cyst is also observed (arrow) (hematoxylin/eosin stain, 100×).

teratoma encompasses patients in all age groups[5].

Scrotal ultrasonography is the mainstay for the preoperative diagnosis of epidermoid cysts and reveals a well-circumscribed, intratesticular lesion with normal surrounding testis. The sonographic features include a sharply defined mass with a hyperechoic rim representing the fibrous cyst wall (Fig. 1, 4), an onion-ring appearance which represents the lamination of the keratin substance within the cyst (Fig 1-4), and alternating hypoechogenic and hyperechogenic areas representing layers of compacted keratin and desquamated squamous cells. A target or bull's eye pattern, indicating the keratinized debris collected centrally in the lesion may be observed (Fig. 1, 2, 5). Retrospective review of the scrotal ultrasonography in the current case had the characteristic findings of an epidermoid cyst. The radiological characteristics of epidermoid cysts of the testes, described previously, correlate well with the histopathological findings[3].

Orchiectomy or testicle-preserving surgery in patients with testicular tumors poses an issue for surgeons. In the absence of reliable clinical or sonographic criteria for differentiating testicular epidermoid cysts, a type of prepubertal teratoma, from malignant testicular tumors, orchiectomy is the first choice that achieves cure by complete excision[4].

In conclusion, in the current case, the "onion peel" and the "target/bull's eye" patterns were observed by both scrotal ultrasonography and gross examination of the excised tumor and correlated well with the histopathological findings (Fig 1-5), emphasizing the importance of the recognition of the characteristic ultrasound findings of intratesticular epidermoid cysts, a type of prepubertal teratoma, for accurate preoperative diagnosis.

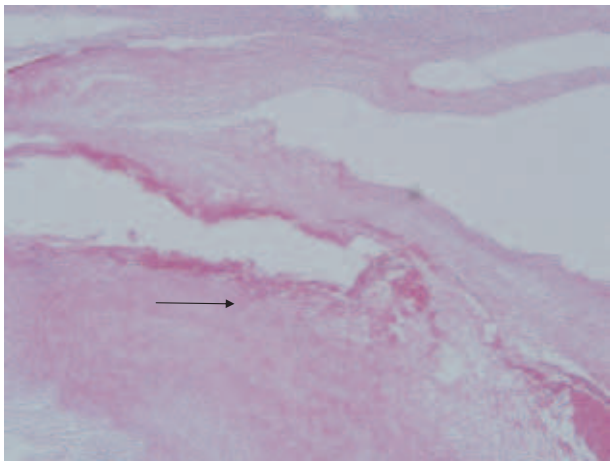

Fig. 5 The cyst lumen contains keratinized debris in the center (arrow) ((hematoxylin/eosin stain, 100×).

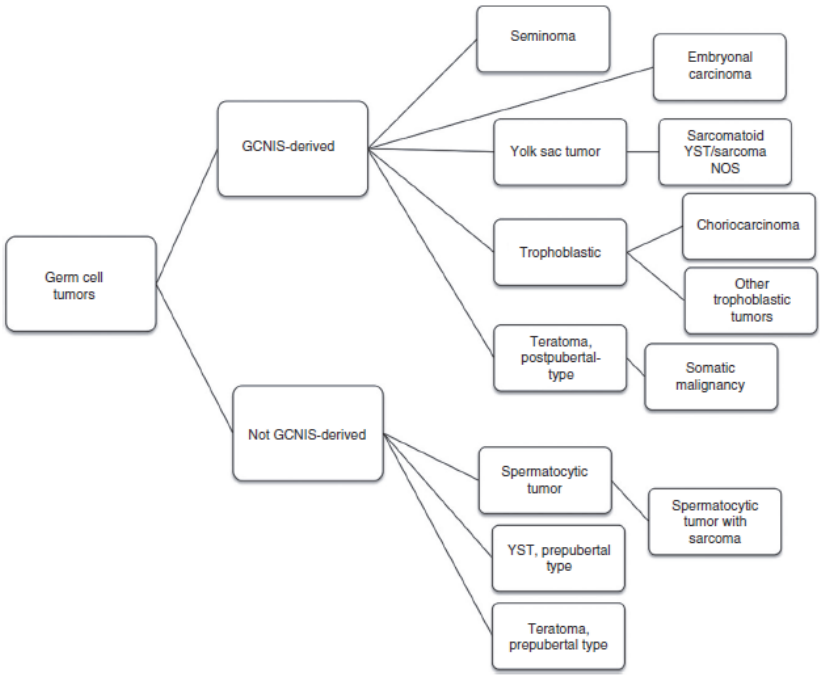

Fig. 6 According to the 2016 edition of the World Health Organization classification, germ cell tumor classification is restructured into tumors derived from germ cell neoplasia in situ (GCNIS) and those not derived from GCNIS. NOS, not otherwise specified; YST, yolk sac tumor (Excerpts from reference 5).

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睪丸內表皮樣囊腫（青春期前畸胎瘤）：陰囊超音波與病理特徵相關性

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摘要

睪丸內表皮樣囊腫（青春期前畸胎瘤）是良性而且少見的睪丸腫瘤，本文報告一位 30 歲男性左邊睪丸有一個腫塊大約有 3 個月時間，理學檢查大約為 2 公分睪丸內不會觸痛的腫塊，超音波檢查發現在左睪丸實質下方有 2.1 公分實質腫瘤 , 無血流量增加現象，而且病人血中 α 胎兒蛋白以及絨毛膜激素均正常，手術前診斷為左側良性睪丸腫瘤，做了睪丸摘除術。本文報告此一少見的睪丸良性腫瘤，討論其陰囊超音波診斷及病理特徵的相關性。

關鍵詞：睪丸內表皮樣囊腫、青春期前畸胎瘤

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Pathology Page

Ewing Sarcoma/Primitive Neuroectodermal Tumor of the Lung

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Abstract

A 79-year-old man presented with shortness of breath and a 10-kg body weight loss in 2 months. Chest computed tomography revealed an irregular margin and hyperdense mass measuring 7.5 cm in diameter in the right upper lobe of the lung. Pre-tracheal, ipsilateral hilar, mediastinal, and subcarinal lymph nodes were enlarged. A biopsy was performed, and the pathological diagnosis was Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET) of the lung. In children and adolescents, ES/PNET is the second most common primary malignant bone tumor, following osteosarcoma. It most frequently arises in long bones. Pulmonary ES/PNET is very rare, and it has been described solely in literature case reports. The present study reports a case of ES/PNET of the lung.

Keywords: Lung tumor, Sarcoma, Small blue round cell tumor, Ewing sarcoma, Primitive neuroectodermal tumor

Pathology Page

A 79-year-old man presented with shortness of breath and a 10-kg body weight loss in 2 months. The patient had a history of hypertension, congestive heart failure, and coronary artery disease for which he received percutaneous coronary angioplasty. The patient was routinely followed up by cardiovascular clinicians of the outpatient department (OPD). Chest computed tomography (CT) revealed a hyperdense mass with irregular margins measuring 7.5 cm in diameter in the right upper lobe of the lung (Fig. 1, CT). Additionally, an enlargement was observed in the pre-tracheal, ipsilateral hilar, mediastinal, and subcarinal lymph nodes.

The patient underwent a biopsy of the lung's tumor located in the right upper lobe. Microscopy findings revealed the following characteristics: sheets of closely packed uniform cells with round uniform nuclei, salt and pepper chromatin, and scanty cytoplasm. Focal Homer Wright rosette formation was identified (Fig. 2, encircled area in the upper panel). Immunohistochemical staining revealed positivity of the tumor cells for both membranous and cytoplasmic CD99 (Fig. 2, lower panel). Additionally, tumor cells were positive for vimentin and bcl-2. Due to negativity for CK, TTF-1, and synaptophysin and chromogranin A staining, small cell carcinoma was ruled out. Furthermore, negative staining for CD3 and CD20 allowed to rule out malignant lymphoma. Similarly, a negative staining for Myo D1 excluded rhabdomyosarcoma. As a consequence, a pathological diagnosis of Ewing sarcoma/primitive neuroectodermal tumor (ES/PNET) of the lung was made.

After this diagnosis, brain CT revealed absence of tumor metastasis. While bone scan, abdominal CT, and PET studies were suggested to the patient, he refused further studies. Additionally, the patient refused both palliative chemotherapy and radiotherapy. He received follow-up at the OPD. The possibility of metastatic ES/PNET to other organs could not be completely ruled out due to insufficient systemic imaging studies. Three months later, the patient

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reported the development of shortness of breath again. CT of the abdomen and chest revealed bilateral lung, liver, spleen, and left kidney metastases as well as pulmonary edema. The patient died due to pulmonary edema and disease progression.

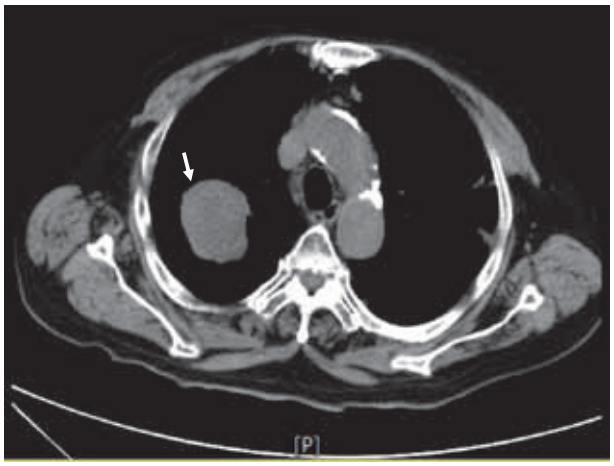

Fig. 1 Computed tomography of the chest. A hyperdense mass with irregular margins measuring 7.5 m (arrow) in diameter can be observed in the right upper lobe of the lung.

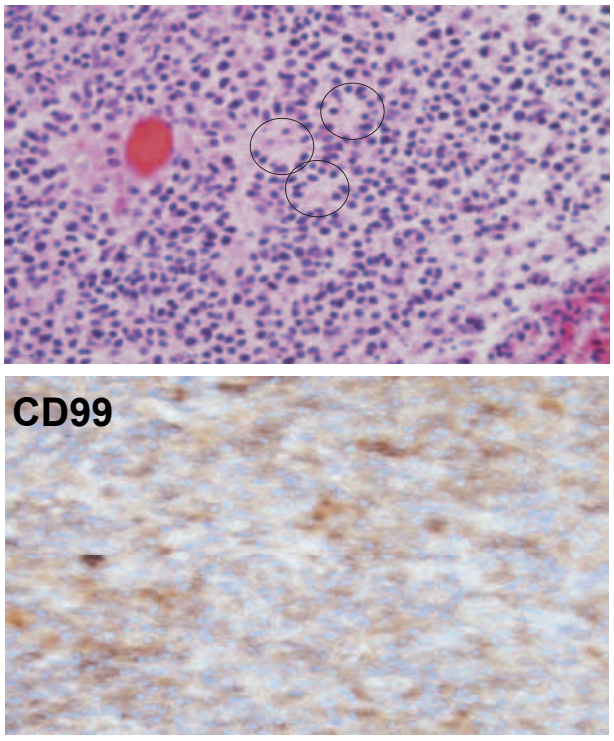

Fig. 2 High magnification of a histopathology showing a sheet of tumor containing closely packed uniform cells with round uniform nuclei and scanty cytoplasm. Focal Homer Wright rosette formation (encircled area) is identified (upper panel, H&E staining, 400×). CD99 immunohistochemical staining was positive in the membrane and cytoplasm of tumor cells (lower panel, 400×).

ES/PNET tumors generally arise in the soft tissue and bone. Tumors may originate from the lungs, hard palate, colon, ovaries, uterus, kidneys, and pancreas. Previous studies have shown several translocations and fusions involving the EWSR1 and ETS family genes. Nearly 90% of the cases are linked to t(11;22) (q24;q12) EWSR1–FLI1 fusion, whereas 5%-10% of cases are associated with t(21;22) (q12;q12) EWSR1–ERG fusion. Pulmonary ES/PNET is a very rare condition. Pathologically, ES/PNET is characterized by sheets of uniform small blue round cells with a scanty cytoplasm. CD99 expression is helpful for the pathological diagnosis of ES/PNET, differentiating it from other small blue round cell tumors (e.g., small cell carcinoma, embryonal rhabdomyosarcoma, malignant lymphoma, myeloid sarcoma, leukemia, and synovial sarcoma). In the present case, CD99 was immunohistochemically expressed in the cell membrane and cytoplasm. Due to the limited number of cases of ES/PNET, treatment guidelines are unavailable. Previously reported cases of primary pulmonary ES/PNET were treated with combinations of surgery, chemotherapy, and radiation therapy. The 5-year survival rate for ES/PNET is approximately 65%–90%. However, patients with metastases at the time of diagnosis have a 5-year survival rate of 25%.

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肺部尤文氏肉瘤 / 神經外胚層母細胞瘤：病例病理報告

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摘要

一位 79 歲男性最近二個月因呼吸困難及體重減輕 10 公斤求診，肺部電腦斷層檢查顯示右上肺葉有一顆 7.5 公分大、高密度且周界不清楚的腫瘤，伴隨有氣管前淋巴結、同側肺門淋巴結、縱膈腔淋巴結、氣管隆凸下淋巴結腫大。病患接受肺腫瘤切片後病理診斷為尤文氏肉瘤 / 神經外胚層母細胞瘤。尤文氏肉瘤 / 神經外胚層母細胞瘤是小孩及青少年第二常見的骨頭腫瘤，僅次於惡性骨肉瘤。本病例報告是一例肺部尤文氏肉瘤 / 神經外胚層母細胞瘤，這在文獻中是非常少見的肺部腫瘤。

關鍵詞：肺腫瘤、惡性肉瘤、小藍圓細胞腫瘤、尤文氏肉瘤、神經外胚層母細胞瘤

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3. 綜論（Review Articles）不必按原著論文格式撰寫，但每篇字數 3500 字以內，摘要 300 字以內，參考文獻 60 篇以內。
4. 短論（Brief Communications），臨床上、技術上的精簡論著，每篇字數 750 字以內，摘要 150 字以內，參考文獻 7 篇以內。

5. 影像判讀（Images）、臨床病理討論（Pathology Page）圖例說明每篇字數 500 字以內，摘要 150 字以內，參考文獻 3 篇以內。
6. 編者的話（Editorials），每篇字數 2000 字以內，摘要 150 字以內，參考文獻 7 篇以內。
7. 其他細節，請參閱國際指導委員會（International Steering Committee）發表之生物醫學雜誌稿件統一規格（Uniform Requirements for Manuscripts Submitted to Biomedical Journals，見 The New England Journal of Medicine 336:309-315,1997）。
8. 將可接受投稿之稿件種類之摘要字數、字數、參考文獻及圖表相關上限規定，整理於下表：

稿件種類	字數限制		參考文獻	圖 / 表
	摘　　要	內文字數		
原著論文 (Original Article)	≦ 300	≦ 3000	≦ 40	≦ 5
病例報告 (Case Report)	≦ 150	≦ 1500	≦ 10	≦ 3
綜論 (Review Article)	≦ 300	≦ 3500	≦ 60	≦ 6
短論 (Brief Communication)	≦ 150	≦ 750	≦ 7	≦ 1
影像判讀 (Images)、 臨床病理討論 (Pathology Page)	≦ 150	≦ 500	≦ 3	≦ 2
編者的話 (Editorial)	≦ 150	≦ 2000	≦ 7	≦ 1

參、投稿須知

1. 稿件須符合「生物醫學雜誌投稿之統一規定」[1]，請以電腦隔行 double space 書寫，並編行號及頁碼，中文字型以標楷體，英文字型以 Time New Roman 12 號字大小，稿紙之左右緣爲 2.54 公分，上下緣爲 3.17 公分。
2. 第一頁爲標題頁，須列出中文及英文之論文題目、中英文作者姓名、所屬機構及單位之中英文稱號（分屬不同單位，請以阿拉伯數字標出作者與單位）、聯絡人姓名、電話及中英文通訊錄。
3. 第二、三頁爲中文及英文之摘要及關鍵詞（請提供 3 至 5 個關鍵詞或簡短片語），中英文摘要須完全相同，摘要分段撰寫，依序爲背景及目的（Background and purpose）、方法（Methods）、結果（Results）及討論（Discussion）。
4. 相同貢獻作者請加註說明，如研究主題的設定、參與決定研究設計、進行統計分析、詮釋研究結果、以及各章節撰稿等貢獻。
5. 圖表應專業製作，一張紙僅一個附圖或附表，依引用順序以阿拉伯數字標出排列。附表須有標題及說明且不可以照片形式。圖片或照片電子檔（.jpg）必須清晰、分明。附圖須有簡單說明（Legend），並另頁撰寫。光學或電子顯微鏡照片，請註明擴大倍率或比例。

註：[1] 根據「生物醫學雜誌投稿之統一規定」第五版，刊載於 Annals of Internal Medicine 1997;126(1): 36-47.

肆、參考文獻

未經發表之論文或摘要不得列爲參考文獻，但可於本文中說明並註明「未發表」（unpublished observations）。博碩士論文可引用。已被任何雜誌接受刊發但仍未發表之著作，請列出雜誌名稱及年份，並註明「in press」。

原著論文、病例報告、綜論、短論、影像判讀、臨床病理討論、編著的話按下列格式撰寫：

A.雜誌及期刊

中文例〔作者姓名：題目 。雜誌簡稱 年號；卷數：起訖頁數〕

薛玉梅、陳建仁：皮膚砷癌之流行性病學特徵與危險因子。中華衛誌 1996; 15: 1-26。

英文例〔英文原稿中引用的參考文獻，其雜誌或期刊之簡稱應參照 Index Medicus 型式〕

1. Feely J, Wilkinson GR, Wood AJ. Reduction of liver blood flow and propranonol metabolism by cimetidine. N Engl J Med 1981;304:691-6.
2. Kaplan NM. Coronary heart disease risk factors and antihypertensive drug selection. J cardiovasc Pharmacol 1982; 4(suppl 2): 186-365.（引用雜誌附冊時）
3. Tada A, Hisada K, Suzuki T, Kadoya S. Volume measurement of intracranial hematoma by computedtomography. Neurol surg (Tokyo) 1981; 9: 251-6.〔In Japanese: English abstract〕（引用文獻之作者之本文爲非英文，但有英文摘要）。
4. Bhasin S, Storer TW, Berman N, Callegari C, Clecenger B, Phillips J, et al. The effects of supraphysiologic doses of testosterone on muscle size and strength in normal men. N Engl J Med 1996; 335: 1-7.（作者超過 6 位時，只須列出前 6 位，其它以「等」(et al) 代替）

＊期刊若有「數位物件識別碼 (digital object identifier, DOI)」，則於文獻末。

＊＊內文文獻標示以中括號、數字、上標呈現。

B.單行本：

中文例 [作者姓名：書名，版數（卷數）。發行地；出版公司，年代：引用部份頁數]。

楊志良：生物統計學新論，一版。台北；巨流圖書公司，1984：33-8.

英文例 [英文單行本的書名，除介系詞及連接詞外，第一字母需大寫]

(1) Plum F, Posner JB. Diagnosis of Stupor and Coma. 3rd ed., Philadelphia: Davis, 1980:132-3.

C.多重作者之單行本：

中文例 [有關文章作者姓名：題目 。編輯者姓名：書名 。版數（卷數）。發行地：出版公司，年代；引用部份頁數]。

蔣欣欣：護理與健康。顧乃平：護理專業導論。一版。台北：匯華出版公司，1991：83-121。

英文例 Levinsky NG: Fluid and electrolytes. In: Thorn GW, Adams RD, Braunwald E, lsselbacher K, Petersdprf RG eds. Harrison’s Principles of Internal Medicine. 8th ed. New York: Mcgraw-Hill, 1977;364-75.

D.參考文獻引用時，若兩名以下作者請列出姓氏。兩名以上則列出第一名之姓氏，其他以「等」(et al) 代替，並以阿拉伯數字方括弧表示於引用之後。

例：One of the first well documented reports of ECH poisoning with fatality in young children was reported by Miller et al. in 1970[2].

E. 參考文獻引用網路資料請列出文獻名稱及出處以及引用時間

(Accessed Month day, 2016, at http://www.house.gov/xxxx/min/inves_xxx/index_accord.htm.)

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綜　論

原　著

病例報告

臨床病理討論

影像判讀

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