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# Tungs' Medical Journal

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## IMAGE

Editorial

# Influenza Vaccination Reduced the Incidence of Guillain-Barre Syndrome in the Elderly in Taiwan

Hueng-Chuen Fan[1,2,3], Der-Shiun Wang[4,5], Yuan-Chih Su[6], Chia-Hung Kao[6,7,*]

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**Abstract**

Influenza is a serious threat to humans and has major health and economic impact globally; therefore, numerous countries have conducted a mass immunisation program against seasonal influenza. However, diseases such as Guillain-Barré syndrome (GBS) are regarded as potential adverse effects of influenza vaccination. GBS is a peculiar neurological illness, a postinfectious disease triggering immune dysregulation leading to neurological dysfunction. Could a temporal link between vaccine exposure and a subsequent adverse event indicate that the vaccine directly caused the event? Identifying such a link could cause public panic and result in increased morbidity and mortality owing to decreased vaccination. Analysing comprehensive electronic medical records of Taiwan National Health Insurance (NHIRD) claims and Taiwan Centres for Disease Control (Taiwan CDC) databases, Huang et al. determined the local background incidence of GBS after influenza vaccination. Although these unprecedented values provide evidence of vaccination safety and contribute to global health policies, whether influenza vaccination can protect against vaccination-related GBS was not addressed in Huang's report. To highlight this issue, we reanalysed the NHIRD and Taiwan CDC databases and discovered that H1N1 vaccination not only can protect from influenza and influenza-associated critical conditions; it can also decrease the incidence of influenza and vaccination-related GBS, especially in people older than 65 years. Hence we recommend that the vaccine should be administered to individuals older than 65 years.

**Key words:** influenza, vaccination, Guillain-Barré syndrome

Influenza is a serious threat to humans and has major health and economic impact globally. Many countries have conducted mass immunisation for seasonal influenza strains. Diseases such as Guillain-Barré syndrome (GBS) are viewed as a consequence of influenza vaccination because of the excessive number of GBS cases that occurred during the mass vaccination campaign against a new strain of influenza in the USA[1]. Although a temporal link between vaccine exposure and a subsequent adverse event does not mean that the vaccine directly caused the event, uncovering such links could inevitably cause public panic and consequently increase morbidity and mortality owing to decreased vaccination. As inappropriate assessment of data regarding vaccine safety may disturb the estimation of the effectiveness of mass vaccination campaigns against influenza, Black et al. emphasised the importance of developing locally relevant background incidence of GBS to aid in the assessment of H1N1 vaccine safety[2]. Analysing comprehensive electronic medical records of Taiwan National Health Insurance (NHIRD) claims and Taiwan Centres for Disease Control (Taiwan CDC) databases, Huang et al. determined the local background

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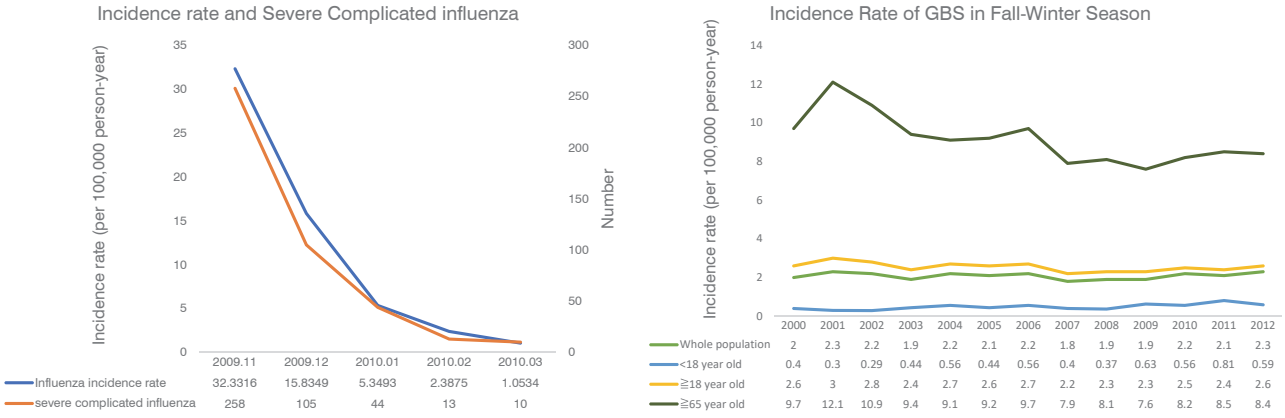


**Fig. 1** (A) Incidence of influenza (blue line) and influenza with severe complications (red line) from November 2009 to March 2010. (B) Incidence of Guillain-Barre Syndrome in fall and winter every year from 2000 to 2012. [deep green line: elderly (≧65 years); light green line: whole population; yellow line: adult (≧18 years); blue line: children (<18 years)]

incidence of GBS after influenza vaccination[3]. These unprecedented values provide evidence of vaccination safety and contribute to global health policies. Whether influenza vaccination can protect against influenza vaccination-related GBS was not addressed in the report of Huang et al[3]. To investigate this issue, we reanalysed the databases of the NHIRD and Taiwan CDC to determine the relationship between the incidence of influenza and severe, complicated influenza (Fig. 1A). Based on the data, we concluded that both influenza and the complications of severe influenza clearly decreased after 1 November 2009, when Taiwan began mass H1N1 vaccination. Furthermore, although the incidence of GBS after influenza vaccination did not decrease yearly in the young people group, it decreased in other groups, most notably in the elderly population (age ≥65 years, Fig. 1B). Our results demonstrate that influenza vaccination is protective against influenza and vaccination-related GBS. The vaccine should highly be recommended and freely administered, particularly to individuals older than 65 years.

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# 施打流感疫苗能夠降低台灣老人格林—巴利式綜合症候群的發生率

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**摘要**

流感是對人類有嚴重的威脅，對於全世界具有重大的健康和經濟影響，因此，許多國家對於季節性流感進行了大規模疫苗接種計畫。格林 - 巴利症候群（Guillain-Barré syndrome; GBS）是一種特殊的神經疾病，它的病因可能是因為像流感等疾病的感染後所引發的免疫異常連帶影響到神經功能；也可能是疫苗接種後所產生的許多不良反應之一。但把 GBS 與流感疫苗的關聯性的過度解讀與渲染，不只會引發大眾對疫苗的恐慌，還可能會引發疫苗接種的減少而導致增加新型流感的發病率和死亡率。儘管 GBS 確實是在疫苗接種後就接踵而來的產生，但這種短暫的關聯性，是否就能意味著疫苗就是直接導致 GBS 的主因呢？藉由分析台灣衛生福利部中央健康保險署（NHI）和台灣疾管局（CDC）資料庫中的資料，CDC 黃醫師推估出流感疫苗接種後可能引發 GBS 的台灣本土背景發生率。此前所未有的數據不但證實了流感疫苗接種的安全性，也有助於全球衛生政策的推展。然而此報告並沒有提到流感疫苗接種是否真能有效減少流感疫苗有關的 GBS。為了釐清此問題，我們再分析台灣衛生福利部中央健康保險署和台灣 CDC 資料庫，發現流感疫苗接種不僅可預防流感，減少流感重症的發生，也可對抗疫苗接種相關的 GBS，特別是在大於 65 歲的老年人，因此我們呼籲民眾特別是老年人最好接種流感疫苗。

**關鍵詞：**流感、疫苗、格林 - 巴利症候群

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Review Article

# The Association Between Antiepileptic Drugs and Bone Health

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**Abstract**

Bone and calcium metabolism disorders are emerging as major issues in the treatment of pediatric patients with epilepsy because children in this stage experience rapid growth, weight gain, and skeletal and genital maturation under the influence of several hormones. There is a relatively complex and multifaceted relationship among epilepsy, antiepileptic drugs (AEDs), and bone and calcium homeostasis. Whether classical AEDs (e.g., benzodiazepines, carbamazepine, phenytoin, phenobarbital, and valproic acid) or newer AEDs (e.g., levetiracetam, oxcarbazepine lamotrigine, topiramate, gabapentin, and vigabatrin) can adversely affect bone health remain unclear. Cytochrome P450 induction, which is one of the properties of AEDs, can cause vitamin D deficiency and lead to lower calcium levels, lower bone mass, increased fracture risk, and altered bone turnover. However, controversial results have been obtained because of the wide spectrum of the antiseizure activities of these AEDs with several different mechanisms of action, which are discussed in this review.

**Key words:** Bone, calcium metabolism, epilepsy, antiepileptic drugs, cytochrome P450

## Introduction

In addition to determining the height, bones also protect the vital organs and provide support for movement. Although stiff and unyielding, bones are living tissues that are continuously remodeled throughout a person's life. Constant remodeling renews the skeleton and modulates calcium and phosphorus homeostasis. This is critical for the specialized cells engaged in bone remodeling and turnover processes, such as in osteoblasts (initiate bone formation), osteocytes (monitor bone mechanical stresses), and osteoclasts (absorb bone)[1]. These processes are intricately modulated by several hormonal factors that are highly dependent on the phase of the life cycle. The rate of annual calcium turnover is 100% in infants and 18% in adults. Up to 95% of total bone development is completed by the age of 18 years[2]. Therefore, childhood and adolescence are most critical periods during which bone is accrued. Any factor interfering with bone homeostasis may affect the bone health.

Epilepsy is one of the most frequently occurring neurological disorders, affecting approximately 50 million people worldwide. The estimated average prevalence of epilepsy in the US is 6.8 per 1000 people, compared with 5.5 per 1000 people in Europe, 1.5–14 per 1000 people in Asia, and 2.8 per 1000 people in Taiwan[3, 4]. As part of the management of patients with seizures, clinicians should perform a thorough medical evaluation including a detailed description of the seizure semiology; the assessment of present, past, and family history; physical examination; laboratory analysis; awake and sleep electroencephalograms; and brain magnetic resonance imaging. Appropriate classification of the seizure type and epileptic syndrome is essential in

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the treatment of epilepsy. Although numerous alternative treatment options are available for epilepsy, including vagus nerve stimulation, epilepsy surgery, and ketogenic diets, approximately 50% of patients with newly diagnosed epilepsy are successfully controlled with the use of adequate antiepileptic drugs (AEDs)[5]. Hence, AEDs represent the primary treatment of choice.

Skeletal disorders are reported in patients with epilepsy, and restrictions of physical activity imposed by seizures or coexisting neurologic deficits, inadequate sunlight exposure, and poor diet appear to be the causes. To date, it is difficult to conclude whether the changes in bone and calcium metabolism are attributable to epilepsy itself or the use of AEDs. Some of these changes are, at least to some extent, AED-related. AED administration has been continually linked to adverse effects on bone health and affect patients of both sexes and at all ages[6]. Classical AEDs such as benzodiazepines (BZDs), carbamazepine (CBZ), phenytoin (PHT), phenobarbital (PB), and valproic acid (VPA) are reported to decrease bone mineralization, and their ability to degrade vitamin D by inducing cytochrome P450 (CYP) isoenzymes is believed to the main mechanism[7]. Newer AEDs, such as levetiracetam (LEV), oxcarbazepine (OXC), lamotrigine (LTG), topiramate (TPM), gabapentin (GBP), and vigabatrin (VGB), have been developed to improve the treatment of refractory seizures as well as the tolerability and safety. However, both classical and newer AED can cause adverse bone effects[6]. Clinicians should pay more attention to the use of AEDs in children and adolescents because of the rapid growth in this period and the particular vulnerability of these age groups to effects on the skeleton.

Numerous techniques have been used to obtain histologic and radiographic evidence of bone abnormalities in patients receiving AEDs, ranging from bone biopsies to dual-energy X-ray absorptiometry (DXA), the present gold standard for detecting decreases in *bone* mineral density (BMD). Early reports suggested that 65–84% of patients with epilepsy who are receiving AEDs develop signs of bone abnormalities, such as rickets, osteomalacia, osteoporosis, and increased fracture risk[6, 8]. Rickets is a disorder of defective mineralization of cartilage in the epiphyseal growth plates of children, leading to widening of the ends of long bones, growth delay, skeletal deformities, and delayed developmental milestones. Bone biopsy reveals the accumulation of unmineralized bone, and biochemical findings include low calcium, phosphorous, and vitamin D metabolite levels and elevated alkaline phosphatase content[9]. Osteomalacia is a metabolic disorder caused by deficiency of vitamin D or its metabolites, which leads to the failure of mature bone to mineralize and the eventual softening of bones. Drug-induced osteomalacia occurs secondary to either deficiencies in calcium, phosphate, and active vitamin D or interference with their deposition or activity. Osteomalacia is linked to signs such as diffuse bone pain, muscle weakness, and bone fragility[10]. Osteoporosis is a disease characterized by low bone mass and reduced bone quality, leading to increased bone fragility and risk of fracture, particularly of the hips, spine, and wrists. Often, there are no symptoms until the first fracture occurs[11]. Apart from eliminating other causes of medical illness, particularly malabsorption, renal disease, and hepatic disease, AED-induced skeletal diseases should be considered in patients receiving AEDs for epilepsy who experience bone pain, muscle weakness, fractures after minimal trauma, or worsening of seizure control. Failure to recognize these issues may lead to the deterioration of patients' conditions.

BMD reflects a complex, dynamic, highly orchestrated process between bone resorption by osteoclasts and the bone-formative action of osteoblasts. A higher of BMD demonstrates the bone in the healthy milieu. Routine X-rays can identify bone fractures, but they cannot not measure bone density. DXA is a low-radiation X-ray technique capable of detecting small percentages of bone loss, and it is the gold standard for measuring BMD. DXA can measure the bone density of the whole skeleton, and the most common sites for clinical use are the spine and hips. Apart from the advantage of detecting minor bone changes, DXA can identify patients at risk of future osteoporosis and provide effective information for protecting against further bone loss. Little is known about the mechanisms by which AEDs cause unfavorable bone changes. Several studies using DXA to measure BMD in patients receiving AEDs for epilepsy revealed significantly reduced BMD at the ribs, spine, femoral neck, and hips and reduced BMD at axial and appendicular sites in children[6, 7]. Markers of bone turnover including markers of bone formation and resorption, which usually increase in osteoporosis, are elevated

in persons with epilepsy who are receiving AEDs[6, 12]. Therefore, these results raise serious concerns about the bone disorders and calcium metabolism of patients with epilepsy who use AEDs and highlight the need to closely monitor growth in children and adolescents with epilepsy. Physicians should be aware of these issues.

The aim of this paper was to provide physicians a short review concerning the impact of classical and newer AEDs on skeletal diseases and calcium metabolism and emphasize the need for a much higher index of suspicion of this entity to ensure timely withdrawal of AEDs and appropriate therapy to avoid serious disabilities.

BZDs are common psychoactive drugs that are widely used to treat anxiety, insomnia, agitation, seizures, status epilepticus, acute repetitive seizures, muscle spasms, alcohol withdrawal, and premedication for some medical or dental procedures. The main effects of BZDs include the enhancement of levels of the neurotransmitter gamma-aminobutyric acid (GABA) and GABA-A receptor-mediated chloride conductance, leading to sedative, hypnotic, anxiolytic, anticonvulsant, and muscle-relaxant effects[13, 14]. The most commonly used BZDs are diazepam, lorazepam, and clonazepam. The core chemical structure of BZDs is a benzene ring fused to a diazepine ring, and their advantages in clinical use include high efficacy rates, a rapid onset of action, and minimal toxicity. Reports revealed that BZDs increase the risk of fractures, and a positive correlation between fracture risk and increasing dose, especially regarding the spine, through mechanisms including reduced BMD, reduced 25OHD, and increased alkaline phosphatase (ALP) levels has been described[12, 15-17]. Conversely, BZD-related bone disorders are not related to the levels of total calcium, phosphorus, magnesium, and parathyroid hormone (PTH); however, controversial results have been reported[8, 18].

CBZ, an iminodibenzyl derivative, is a commonly used medication for partial seizures and secondary generalized seizures in adults and children. More than 90% of countries list CBZ as an essential drug for their populations' health. CBZ is extensively metabolized in the liver, and the main metabolite is CBZ-10, 11-epoxide, which possesses anticonvulsant properties. Additionally, CBZ mainly acts on voltage-gated sodium channels that are stabilized in their inactivated state, reduces polysynaptic responses, and blocks post-tetanic potentiation. CBZ may cause several adverse effects, including sedation, ataxia, dizziness, nausea, vomiting, constipation, diarrhea, altered metabolism of lipids, changes in sex hormone levels, hyponatremia, weight gain, anemia, agranulocytosis, and allergic reactions[6, 19]. CBZ is reported to cause spina bifida in neonates exposed to the drug in utero[20]. Several studies revealed that CBZ can decrease BMD in the lumbar spine, femoral neck, and forearms. That is because CBZ can induce CYP isoenzymes, possibly reducing vitamin D levels[21, 22]. Additionally, high levels of markers of bone formation (bone ALP, osteocalcin, carboxy-terminal propeptide of type I procollagen, and amino-terminal propeptide of type III procollagen) and bone resorption (carboxy-terminal telopeptide of type I collagen and the

BZDs

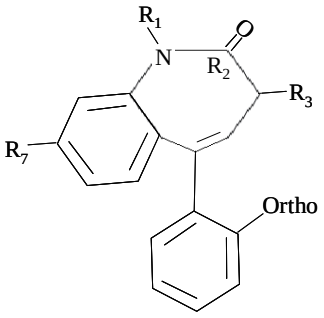


**Fig. 1** Structural formula of 1,4-benzodiazepines.

CBZ

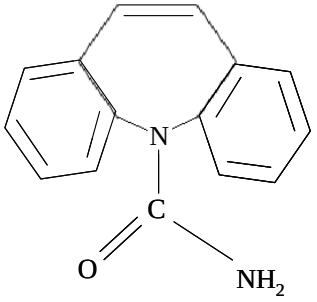


**Fig. 2** Structural formula of carbamazepine.

PHT

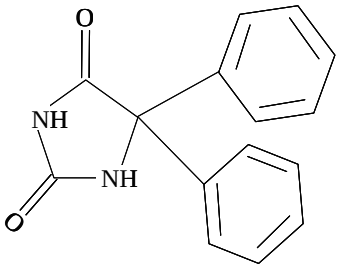


**Fig. 3** Structural formula of phenytoin.

urinary cross-linked N-telopeptides of type I collagen [NTx]) were detected patients receiving CBZ, but their vitamin D levels were normal[12]. Interestingly, CBZ may have a direct effect on bone cell proliferation, leading to reduced growth of human bone cells[23].

PHT is a hydantoin that was identified to have hypnotic effects on electroshocks inducing seizures in cats. PHT is used to treat generalized tonic–clonic seizures and status epilepticus, and its primary pharmacological effects possibly include promoting $Na^+$, $K^+$, and $Ca^{2+}$ ion conductance, reducing membrane action potential, and altering amino acid concentrations to block hyperexcitability caused by excessive stimulation or environmental changes capable of reducing the membrane electrochemical gradient rather than increasing the seizure threshold and abolishing the primary focus of discharge[24]. The bioavailability of oral PHT is high, but the time to peak blood levels ranges from 3 to 12 h. The half-life of PHT is 12–36 h. Reports found that patients who receive PHT for epilepsy may have reduced lumbar spine, femur, and hip BMD[17]. PHT induces CYPs, leading to increased catabolism of vitamin D and eventual hypocalcemia[25]. PHT interferes with intestinal calcium absorption in rats, leading to lower calcium levels in serum[26]. Animal studies also revealed a direct inhibitory effect of PHT on calcitonin secretion, which affects bone resorption and formation[23, 27-29]. Patients treated with PHT exhibited elevated levels of markers of bone turnover in the serum, including markers of bone formation and bone resorption[23, 27, 28, 30, 31]. Clinically, patients with hyperparathyroidism display biochemical abnormalities, including higher PTH, ALP, and urinary NTx levels, and histological findings such as osteomalacia, suggesting that hyperparathyroidism can primarily activate bone resorption. An in vitro study uncovered that fetal rats treated with PHT exhibited an impaired osteoblastic response to PTH[23], suggesting the drug directly modulates bone metabolism. In conclusion, although there are some contradictory results regarding the links of PHT with adverse effects on bone mineralization and an increased risk of fractures, accumulating evidence illustrated that PHT can induce several abnormalities in bone metabolism including hypocalcemia, hypophosphatemia, reduced serum levels of biologically active vitamin D metabolites, hyperparathyroidism, and elevated levels of markers of bone turnover. Therefore, monitoring the bone mineral status and prophylactic prescription with vitamin D to patients with high bone fracture risk due to anticonvulsant therapy may prevent the possible development of bone disease.

PB, a compound containing a perhydropyrimidine ring substituted at C-2, C-4, and C-6 by oxo groups, has been used since the early twentieth century because it effectively reduces anxiety, promotes sleep, induces general anesthesia, and inhibits tonic–clonic seizures[32]. PB enhances GABA-mediated increases in chloride conductance by prolonging the duration of channel opening, leading to the effective control of seizures. The World Health Organization recommends PB as a first-line treatment for convulsive seizures in resource-poor countries[33]. Studies have found that PB can increase the risks of rickets and hypocalcemia, resulting from decreases in vitamin D levels due to CYP inhibition, impaired intestinal calcium transport, and increased PTH levels. Consequently, bone mineralization was reduced, and osteomalacia developed[34, 35]. However, several studies evaluating vitamin D levels in ambulatory patients reported conflicting results[17, 35-37].

VPA (2-propylpentanoic acid) was originally synthesized as an analog of valeric acid extracted from Valeriana officinalis[38]. VPA is a broad-spectrum AED against several seizure types, including absence,

PB

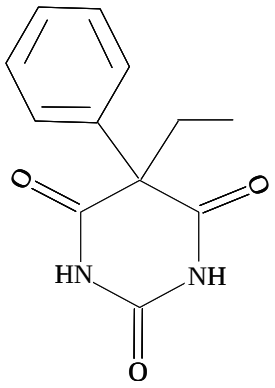


**Fig. 4** Structural formula of phenobarbital.

VPA

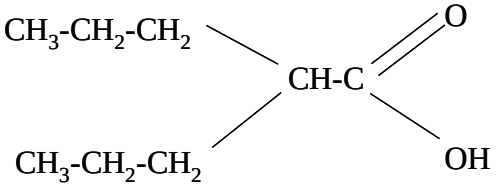


**Fig. 5** Structural formula of valproic acid.

partial, and tonic–clonic seizures, and it is also effective against a variety of psychiatric and neurological diseases such as bipolar disorder, antidepressive effects, migraine, personality disorders, mental disability, dementia, and cognitive problems, in addition to use as a potential chemotherapeutic agent[38, 39]. VPA is reported to elevate GABA levels in plasma and in several brain regions by affecting the GABAergic system, inhibiting succinate semialdehyde dehydrogenase and GABA transaminase, upregulating glutamate decarboxylase, affecting cerebral metabolism by inhibiting alpha-ketoglutarate dehydrogenase in the TCA cycle, and activating GABA receptors[38-40]. VPA can block sodium channels and modulate calcium and potassium conductance and dopaminergic and serotoninergic transmission[38-40]. The effect of VPA on the regulation of glutamate excitatory neurotransmission and/or GABA inhibitory neurotransmission is one of the main mechanisms of its "mood-stabilizing" effect and its effects in the treatment of migraine. GABA-mediated responses, as well as the ability of BZD to prolong the decay time of the post-synaptic inhibitory response by interacting with the BZD regulatory site of the GABA-A receptors and increasing baclofen binding to GABA-B receptors, may be involved in the beneficial effects of VPA on neuropathic pain. VPA is an effective inhibitor of histone deacetylases, the key enzymes for controlling histone acetylation and hence the epigenetic regulation of gene expression, leading to the modulation of cell growth, differentiation, and apoptosis and thereby providing novel strategies for regulating neuroprotective genes and excitotoxicity and treating tumors[41]. Pharmacodynamically, VPA is well absorbed in all oral dosage forms, with greater than 90% bioavailability. Structurally, VPA is related to free fatty acids, and it is highly ionized at physiological pH; therefore, it exhibits a high degree of binding to plasma proteins. VPA is metabolized via microsomal glucuronidation, mitochondrial beta-oxidation, and CYP oxidation[6, 40]. The most common side effects of VPA are nausea, vomiting, abdominal cramps, diarrhea, body weight gain, impaired coagulation system, and neutropenia. Its serious adverse effects include hepatotoxicity, pancreatitis, teratogenicity, endocrine disturbances such as menstrual abnormalities, increased total testosterone levels, and obesity. VPA is reported to decrease[42], not change[43], or increase bone turnover[44-46]. Likewise, the reported effects of VPA on cultured bone cells range from inhibition of both osteoblast and osteoclast activity[47] to enhancement of osteogenesis[48]. Results by Wu et al.[49] and our group[50] suggest that VPA has direct effects on bone growth. In vivo studies investigating the effect of VPA on bone metabolism are limited. In view of the diverse molecular and cellular reactions underlying several seizure types and diseases, VPA possibly possesses distinct neurochemical and neurophysiological characteristics, resulting in a wide spectrum of actions against seizures and neurological diseases. However, the possible occurrence of serious side effects, teratogenesis, and liver toxicity has prompted a search for newer AEDs with better efficacy and fewer side effects.

LEV, the α-ethyl analog of piracetam, has linear pharmacokinetics, minimal protein binding, and a rapid onset of action, and it is completely excreted by the kidneys without any hepatic metabolism. LEV does not interact with other drugs, and it can be administered intravenously or orally twice a day[51], making it safe and effective in the treatment of patients with epilepsy, including those with partial seizures, resistant partial seizures, or other coexisting medical conditions[52]. LEV is proposed to have several modes of action, such as suppression of negative allosteric modulators of neuronal GABA- and glycine-gated currents, inhibition of voltage-gated calcium channels, reduction of voltage-operated potassium currents, and binding to synaptic vesicle protein 2A[52-55], but the exact mechanism of action is unknown. The effects of LEV on bone mass, biomechanical strength, and bone turnover are controversial. For instance, animal studies found that low-dose LEV in skeletally immature rats may affect longitudinal skeletal growth and fracture risk. LEV may impair the strength of the femoral neck by altering the bone microstructure/architecture. LEV was revealed to affect serum estradiol levels in the same rats, suggesting that long-term use of this drug

New-generation AEDs

LEV

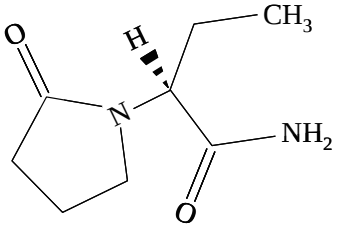


**Fig. 6** Structural formula of levetiracetam.

might increase fracture risk in particularly young and female individuals drug[56]. However, several reports did not observe these adverse effects of LEV[54, 57, 58].

OXC is a structural derivative of CBZ with a ketone in place of the carbon-carbon double bond on the dibenzazepine ring at the 10 position (10-keto). OXC is approved as an adjunctive therapy or monotherapy for the treatment of partial seizures in adults and children. The mechanism of action of OXC is similar to that of CBZ, and it has comparable efficacy but superior safety to its parent drug according to controlled clinical trials[19, 59]. The structural differences may explain the low incidence of CBZ-related allergic reactions, enzyme-induced reactions, anemia, or agranulocytosis. Following oral administration, OXC is rapidly and almost completely metabolized to its monohydroxy derivative (MHD). OXC and MHD both have potent anticonvulsive properties, which are possibly mediated through their effects on neuronal ion fluxes, specifically blockade of voltage-dependent sodium, potassium, and calcium channels[19, 59]. The most common adverse effects of OXC include fatigue, drowsiness, diplopia, dizziness, nausea, vomit, skin rash, and hyponatremia[60]. Two studies reported that decreased BMD was detected in patients on long-term OXC therapy[61, 62]. OXC, which induces CYPs, was found to reduce levels of 25OHD and bone turnover biomarkers, such as PTH and bone ALP, and directly affect osteoblast proliferation, leading to bone loss[23, 62, 63].

LTG is a triazine derivate that can inhibit presynaptic voltage-sensitive sodium channels; block L-, N-, and P-type calcium channels; and inhibit 5-hydroxytryptamine-3 receptors, thereby stabilizing neuronal membranes and inhibiting glutamate release at cortical projections in the ventral striatum limbic areas, consequently leading to reduced GABA levels. LTG can block sustained repetitive firing in cultured mouse spinal cord neurons. Because of these antiseizure advantages and its neuroprotective and antiglutamatergic effects, LTG is approved for the treatment of epilepsy, including simple partial, complex partial, and secondarily generalized seizures, and authorized as an adjuvant therapy for focal onset tonic–clonic, atypical absence, myoclonic seizures, Lennox-Gastaut syndrome (LGS), juvenile myoclonic epilepsy, infantile spasms, absence seizures, and Rett syndrome[64]. LTG is mainly metabolized by the liver, and it is generally well tolerated at its maintenance dose. Although LTG has a broad clinical spectrum of effects, it has several adverse effects, including headache, dizziness, sedation, nausea, insomnia, diplopia, and ataxia. Meanwhile, the incidence of diarrhea and tremor associated with the drug is significantly lower. LTG does not cause weight gain. The incidence of serious rash with LTG treatment was 0.1% in all studies, although the rash can potentially progress to lethal Stevens–Johnson syndrome. To date, reports regarding the adverse effects of LTG on bone diseases are sparse. Guo et al. reported that LTG might impair growth in

OXC

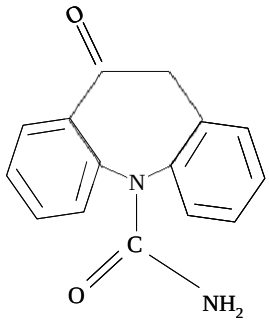


**Fig. 7** Structural formula of oxcarbazepine.

LTG

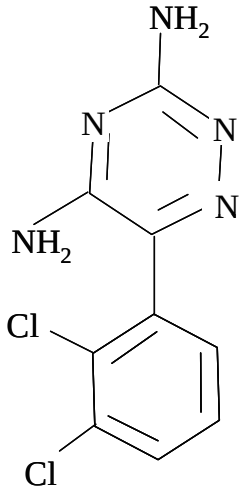


**Fig. 8** Structural formula of lamotrigine.

TPM

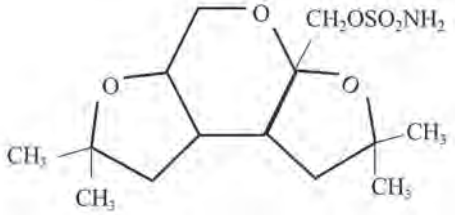


**Fig. 9** Structural formula of topiramate.

children, decrease BMD, and elevate bone turnover markers[43], whereas several groups did not detect any osteopenic effects or significant alterations in bone metabolism in patients on long-term LTG therapy[21, 50, 65].

TPM is a sulfamate-substituted derivative of the monosaccharide D-fructose. Although the precise mechanisms of action are unknown, TPM enhances GABAergic activity, inhibits kainite/alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid-type glutamate receptors and voltage-sensitive sodium and calcium channels, and prevents protein kinases from phosphorylating the channels. Based on these antiseizure properties, TPM is effective as a monotherapy or adjunctive therapy for patients with primary generalized tonic–clonic seizures, partial seizures or seizures associated with LGS[66]. TPM is also useful in the treatment of bipolar disorder, neuropathic pain, depression, obesity, alcoholism, and post-traumatic stress disorder and the prophylaxis of migraine. The common adverse effects of the drug include somnolence, hypo- or an-hydrosis, paresthesia, nystagmus, problems with concentration and word finding, decreased appetite, weight loss, metabolic acidosis, glaucoma, and nephrolithiasis[66, 67]. TPM is a carbonic anhydrase inhibitor). Excessive carbonic anhydrase inhibition may disturb bone metabolism and cause osteomalacia through metabolic acidosis, reducing PTH secretion, impairing the synthesis of 1,25(OH)2D, causing hypocalcemia, and attenuating the activities of osteoclasts[68]. Paradoxically, patients treated with TPM in our previous study did not exhibit significant hypocalcemia[50]. We hypothesized that lower doses of TPM might not disturb the in vivo acid-base homeostasis and the levels of PTH and 1,25(OH)2D, resulting in a subtle change of serum calcium levels.

GBP, a structural analog of GABA, is completely soluble in water and excreted in the urine and feces, but it is not metabolized by the liver. GBP can freely cross the blood–brain barrier, but it does not induce or inhibit hepatic enzymes. GBP is indicated as an adjunctive AED for the treatment of partial seizure with or without secondary generalization in patients older than 12 years and for a variety of pains, including postoperative pain, post-herpetic neuralgia, diabetic neuropathy, complex regional pain syndrome, inflammatory pain, central pain, malignant pain, trigeminal neuralgia, HIV-related neuropathy, and headaches. The mechanisms of action of GBP are complicated. GBP may have an inhibitory effect on voltage-gated calcium channels containing the alpha 2-delta subunit instead of binding to GABA receptors in the central nervous system[69]. GBP may have side effects, including sexual dysfunction and weight gain. The relationship between GBP and bone diseases is not clear. Long-term GBP therapy is proposed to cause bone loss at the hips and lumbar spine[6, 16], and a prospective study concluded that GBP caused significant bone loss in the hips of older men[8], suggesting that it is necessary to closely monitor bone status in patients receiving GBP for epilepsy.

VGB is a GABA-aminotransferase inhibitor that decreases the synaptic breakdown of GABA. VGB is the first-line treatment for infantile spasms secondary to tuberous sclerosis because it is not metabolized by the liver[13]. It is also potentially effective as an initial monotherapy for untreated pediatric partial-onset seizures, LGS, and refractory complex partial seizures in patients with inadequate response to several alternative treatments. Fatigue, headache, dizziness, ataxia, tremor, weight gain, and hyperactivity are commonly noted. However, the drug may aggravate myoclonic seizure and cause serious side effects, such as visual field damage. Periodic ophthalmological examinations are recommended[70]. Although a previous study did not identify significant effects of AEDs, including LTG, TPM, GBP, and VGB, on BMD and bone mineral metabolism[65], VGB was found to decrease body mass gain and inhibit compact bone growth in immature rats[71]. Therefore, VGB should be used with caution. There is no convincing data regarding the relationship between VGB and bone turnover.

GBP

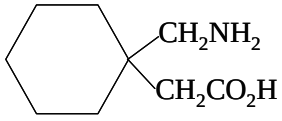


**Fig. 10** Structural formula of gabapentin.

VGB

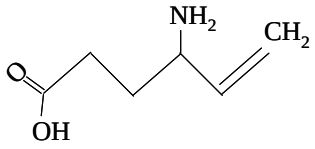


**Fig. 11** Structural formula of vigabatrin.

## Conclusion

Without doubt, AEDs are effective in the treatment of epilepsy. Approximately 50% of patients with newly diagnosed epilepsy become seizure-free after adequate AED treatment. However, bony pathological manifestations, abnormalities in bone and mineral metabolism, and decreased BMD have been commonly reported in individuals treated with classical (BZD, CBZ, PHT, PB, VPA) and/or with newer AEDs (LEV, OXC, LTG, TPM, GBP, VGB). Several mechanisms for AED-associated bone disease have been proposed. Some, but not all, studies proposed that AEDs negatively influence bone health, which was linked to CYP induction, resulting in reduced vitamin D levels and consequently impaired bone growth and calcium metabolism. To date, no single mechanism has successfully addressed all of the reported findings, and no definitive guidelines have been established for evaluation or treatment regarding these adverse effects of AEDs on impaired bone and calcium metabolism. Recent studies suggested that the broad-spectrum effects of AEDs on bone may be multifactorial and potentially injurious. Nevertheless, these results raise serious concerns regarding the bone health of patients who receive AEDs for epilepsy, and a 3–6-month monitoring schedule, prophylactic vitamin D supplementation, sufficient intake of dietary calcium, and weight-bearing exercise are recommended for all patients with epilepsy on initiation of AED therapy.

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# 抗癲癇藥物與骨骼健康的關聯性

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**摘要**

骨骼和鈣代謝疾患是治療兒童癲癇患者的主要問題之一，因為在此階段中，兒童會受到多種荷爾蒙的作用，而有快速生長，體重增加，骨骼和生殖器的成熟。然而在癲癇，抗癲癇藥物，骨骼和鈣平衡之間的關係是相對複雜和多方面。典型的抗癲癇藥物（例如 benzodiazepines, carbamazepine, phenytoin, phenobarbital, 和 valproic acid）或更新的抗癲癇藥物（例如 Levetiracetam, oxcarbazepine lamotrigine, topiramate, gabapentin, 和 vigabatrin）已被報導會對骨骼健康產生不利影響。抗癲癇藥物的另一特性，是細胞色素 P450（CYP-450）同功酶的誘導劑，可能導致維生素 D 的缺乏，鈣的濃度降低，骨量減少，骨折風險增加和改變骨代謝。由於這些抗癲癇藥物有廣泛的抗癲癇作用，涵蓋多種不同的作用機制，造成爭議的結果，這些都將在本文中討論。

**關鍵詞：**骨骼、鈣代謝、癲癇、a 抗癲癇藥物、細胞色素 P450

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Original Article

# Comparison of Post-Ischemic Brain Injury in Young and Middle-Aged Stroke Rats

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**Abstract**

Stroke is a disease of the elderly, and its incidence, disability, and mortality increase with age. Age-associated comorbidity is complex; thus, the exacerbation mechanisms of stroke injury in the elderly are multifactorial. To extend the scope of relevant studies, we aimed to investigate whether age and comorbidities are associated with severe post-ischemic brain injury and identified the factors involved in a rat model of stroke. Male adult Sprague Dawley rats at the age of 12 months (middle-aged) exhibited an endogenous environment that was more conducive to hyperlipidemia, hyperglycemia, hyperinsulinemia, insulin resistance, and chronic low-grade inflammation than rats at the age of 2 months (young). Middle-aged rats suffered from greater exacerbation of brain injury following cerebral ischemia than young rats when both the common carotid and the right middle cerebral arteries were clamped. The metabolic abnormalities of rats rendered them prone to ischemic brain injury, and post-ischemic brain injury in turn further augmented deleterious metabolic abnormalities. In conclusion, our results demonstrate that age-associated comorbidities such as hyperglycemia, insulin resistance, and chronic inflammation predispose rats to cerebral ischemia injury. The mechanisms of ischemic brain injury elucidated in our model of young and middle-aged rats with stroke could serve as the basis for further studies on this condition in the elderly.

**Key words:** Elderly, Inflammation, Metabolism, Stroke

## Introduction

Stroke is a leading cause of mortality and disability worldwide and imposes heavy social and economic burdens. Regardless of the etiologies, effective treatment options for ischemic and hemorrhagic stroke remain limited[1]. Clinically, hypertension, diabetes mellitus, hyperlipidemia, obesity, and smoking are the risk factors for stroke, and it shows high rates of incidence, disability, and mortality[2-4]. Since stroke is mostly a disease of the elderly (>65 years of age), age is the most important independent risk factor for stroke[5]; however, epidemiological evidence indicates that the incidence of stroke in young adults (18–50 years of age) has substantially increased[6]. Thus, it is crucial to gain a better understanding of the mechanisms of stroke and any differences between young and elderly individuals in terms of clinical risk factors.

Rodent models of stroke have been used in preclinical studies to explore pathophysiological mechanisms, disease progression, complications, regeneration, and possible therapeutic options. Rodent

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studies have examined the associations of sex and age with angiogenesis, cognitive impairment, sensorimotor and cerebral electroencephalographic asymmetry, adaptation, and reparative potential has been shown to occur as a result of aging[7-10].

We had previously developed a stroke model using young Sprague Dawley rats by occluding both the common carotid arteries and the right middle cerebral artery, and we found that post-ischemic brain injury was accompanied by sympathetic activation, metabolic abnormality, hyperglycemia, hyperinsulinemia, and insulin resistance, as well as central and peripheral nervous system inflammation[11-14]. To extend the scope of relevant studies, in this study, we aimed to investigate whether age and comorbidities were associated with more severe post-ischemic brain injury, and if so, to identify the factors involved.

## Materials and Methods

### Animals and cerebral ischemia

The Animal Experimental Committee of Taichung Veterans General Hospital approved the animal study protocol (IACUC No. La-101987, Oct. 29, 2012). All experiments were conducted using male Sprague Dawley rats at 2 (young, n = 54) or 12 (middle-aged, n = 54) months of age. Under isoflurane anesthesia, permanent cerebral ischemia was induced by clamping both the common carotid and the right middle cerebral arteries, as described previously[11]. For sham operations, all steps, except for arterial occlusion, were the same. All animals were euthanized at 1 (most assays) or 3 (inflammatory cell assays) days after the surgery and subjected to further analyses.

### Quantification of ischemic infarction

Rats (6 animals/group) were euthanized by isoflurane anesthesia followed by decapitation. Brains were removed and cut into serial coronal sections at 2-mm intervals. First to seventh coronal slices were immersed in a phosphate-buffered saline (PBS) solution containing 2% triphenyltetrazolium chloride at 37°C for 30 min to develop the areas of infarction[11].

### Neurological evaluation

Neurological deficits (6 animals/group) were evaluated using a modified six-point neurological deficit severity scale (0, no neurological deficit; 1, difficulty in fully extending the left forepaw; 2, inability to extend the left forepaw; 3, mild-circling to the left; 4, severe circling to the left; and 5, falling to the left)[11].

### Water content measurement

The contralateral and ipsilateral cortices were isolated (6 animals/group). The tissues were dried in an oven at 110°C for 24 h. The water content was measured using the wet and dry weight method as follows: water content (%) = [(wet weight − dry weight)/wet weight] ×100[11].

### Blood–brain barrier (BBB) permeability measurement

Rats (6 animals/group) were injected with Evans blue (4%, 1 ml/kg) via the tail vein 3 h prior to euthanasia, followed by heparinized saline solution perfusion. The contralateral and ipsilateral cortices were isolated, weighed, homogenized in 500 µl PBS and precipitated with 500 µl of trichloroacetic acid (100%). Evans blue content in the supernatants was quantified (absorbance at 620 nm)[11].

### Plasma biochemical analyses

Rat chow was withdrawn 12 h prior to plasma sample collection. Blood samples were withdrawn from the left femoral artery and stored in liquid nitrogen until analyses. Plasma levels (6 animals/group) of alanine aminotransferase (ALT), blood urea nitrogen (BUN), triglycerides, and total cholesterol were measured via automated standardized procedures (Union Clinical Laboratory, Taichung, Taiwan). Plasma glucose levels were measured using a handheld Accu-Chek glucometer (Roche Diagnostics, Indianapolis, IN, USA), and plasma insulin levels were determined using an enzyme-linked immunosorbent assay (ELISA) kit following the manufacturer's protocol (*Shibayagi,* Gunma, Japan).

### Glucose and insulin tolerance test

To conduct glucose and insulin tolerance tests, fasting rats (6 animals/group) were intraperitoneally injected with a glucose solution (2 g/kg) or insulin (1 U/kg). Blood samples were then collected from tail veins over time to measure the glucose level. The total area under the curve (AUC) for glucose during the intraperitoneal glucose tolerance test (2-h glucose AUC) and the intraperitoneal insulin tolerance test (1-h glucose AUC) was calculated using the trapezoidal (trapezium) rule.

### C-reactive protein (CRP) measurement

Plasma CRP levels (6 animals/group) were measured using an ELISA kit following the manufacturer's protocol (R&D Systems, Minneapolis, MN, USA).

### Identification of white blood cells (WBCs) in blood

Rats (6 animals/group) were anesthetized, and blood samples were withdrawn from the left femoral artery. Blood smears were prepared and stained with Liu's stain. Various types of WBCs were observed and counted under a light microscope. A total of 500 cells were counted for comparison[11].

### Flow cytometry

Inflammatory cells from cortical brain tissues were isolated, prepared, and subjected to flow cytometry cell-type analyses in accordance with our previously reported methods[11]. Inflammatory cells were isolated from the contralateral and ipsilateral cortical tissues (6 animals/group). To determine changes in intracerebral inflammatory response, the number and activation of macrophages/microglia in the rats' brains were analyzed using fluorescence-activated cell sorting analysis after visualization of subtype-specific surface markers by fluorophore-labeled antibodies against CD45 (BioLegend, San Diego, CA, USA) and CD11b (eBioscience, San Diego, CA, USA). Antibody-labeled cells were characterized on a BD FACSCalibur flow cytometer. Data obtained were analyzed using Cell Quest (BD Biosciences, San Diego, CA, USA).

### Statistical analysis

Data are expressed as mean and standard deviation. Statistical analysis was performed using a one-way analysis of variance, followed by Dunnett's test, to assess any statistically significant differences between the treated and untreated groups in all experiments. A $p < 0.05$ was considered significant.

## Results

### Young and middle-aged rats showed distinct metabolic characteristics

Middle-aged rats showed greater body mass and higher plasma levels of triglycerides, total cholesterol, fasting glucose, fasting insulin, and CRP, with the exception of ALT and BUN, than young rats (Fig. 1). Thus, middle-aged rats exhibited the dysregulation of lipid and glucose metabolisms along with systemic inflammation, although liver and kidney functions were normal.

### Middle-aged rats developed more severe stroke injury

Middle-aged rats developed more apparent neurological deficits (Fig. 2A) and brain infarction (Fig. 2B) than young rats. Furthermore, cerebral ischemia led to Evans blue parenchymal extravasation in ipsilateral cortical tissues, with augmented levels in middle-aged rats. Moreover, the Evans blue levels in the contralateral tissues of the middle-aged ischemic rats were higher than those in the contralateral tissues of young rats (Fig. 2C). Moreover, the water

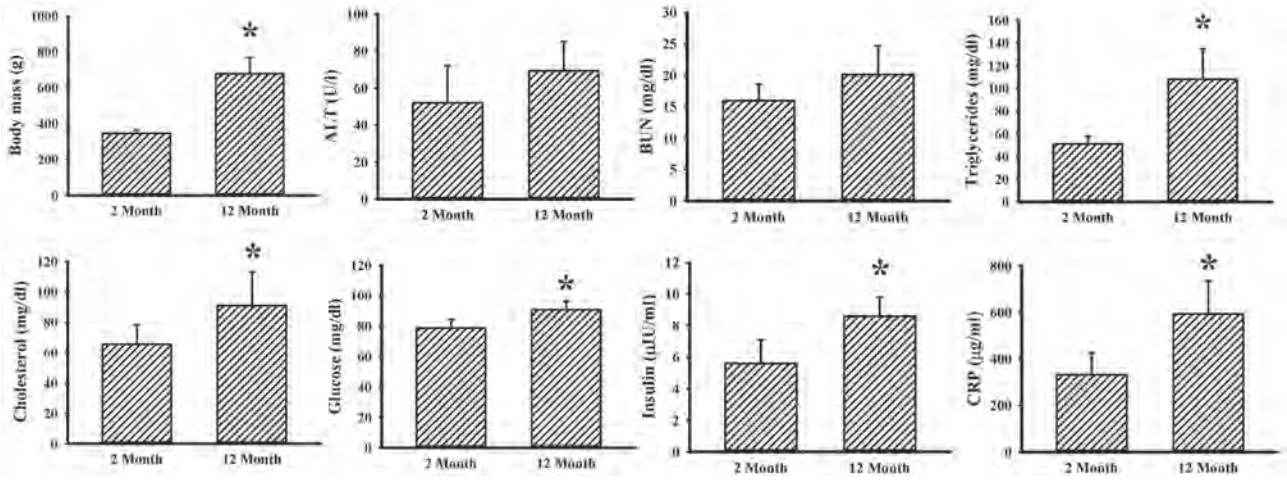


**Fig. 1** Characteristics of young and middle-aged rats. Body mass and plasma levels of ALT, BUN, triglycerides, total cholesterol, glucose, insulin, and CRP were measured in rats aged 2 or 12 months. *$p < 0.05$ vs. 2 months, $n = 6$.

content was elevated in the ipsilateral cortical tissues following ischemia, but there was no apparent difference in the water content of the contralateral tissues between the two groups (Fig. 2D). These results suggest a worse outcome in middle-aged rats following cerebral ischemia.

**Middle-aged rats developed higher glucose and insulin intolerance**

Following cerebral ischemia, middle-aged rats developed more severe hyperglycemia (Fig. 3A) as well as higher glucose (Figs. 3B and 3C) and insulin (Figs. 3D and 3E) intolerance than young rats. These results indicate that dysregulated glucose metabolism is associated with cerebral ischemia injury.

**Middle-aged rats showed increased circulating leukocyte and intracerebral infiltration after cerebral ischemia**

The number of circulating WBCs and percentages of neutrophils and monocytes significantly increased in middle-aged rats compared with those in young rats as well as at 3 days after the onset of ischemia compared with values in each corresponding sham operation group (Fig. 4). Moreover, cerebral ischemia further elevated circulating WBCs and percentages of neutrophils and monocytes in the bloodstream of middle-aged rats (Fig. 4). The percentages of $CD45^{+}/CD11b^{+}$ macrophages/microglia (Fig. 5A) and activated macrophages/microglia ($CD45^{high}/CD11b^{high}$) (Fig. 5B) in the contralateral and ipsilateral cortical tissues were not significantly different between the sham groups of young and middle-aged rats. Cerebral ischemia increased the percentage of total macrophages/microglia (Fig. 5A) and activated macrophages/microglia (Fig. 5B) in the ipsilateral cortical tissues of young rats compared with those in the ipsilateral cortical tissues of rats in the corresponding sham groups. However, cerebral ischemia worsened in both the contralateral and ipsilateral cortical tissues of middle-aged rats, with greater exacerbation in the latter tissue type than in the former (Figs. 5A and 5B). These findings suggest that middle-aged rats show elevated circulating leukocytes and

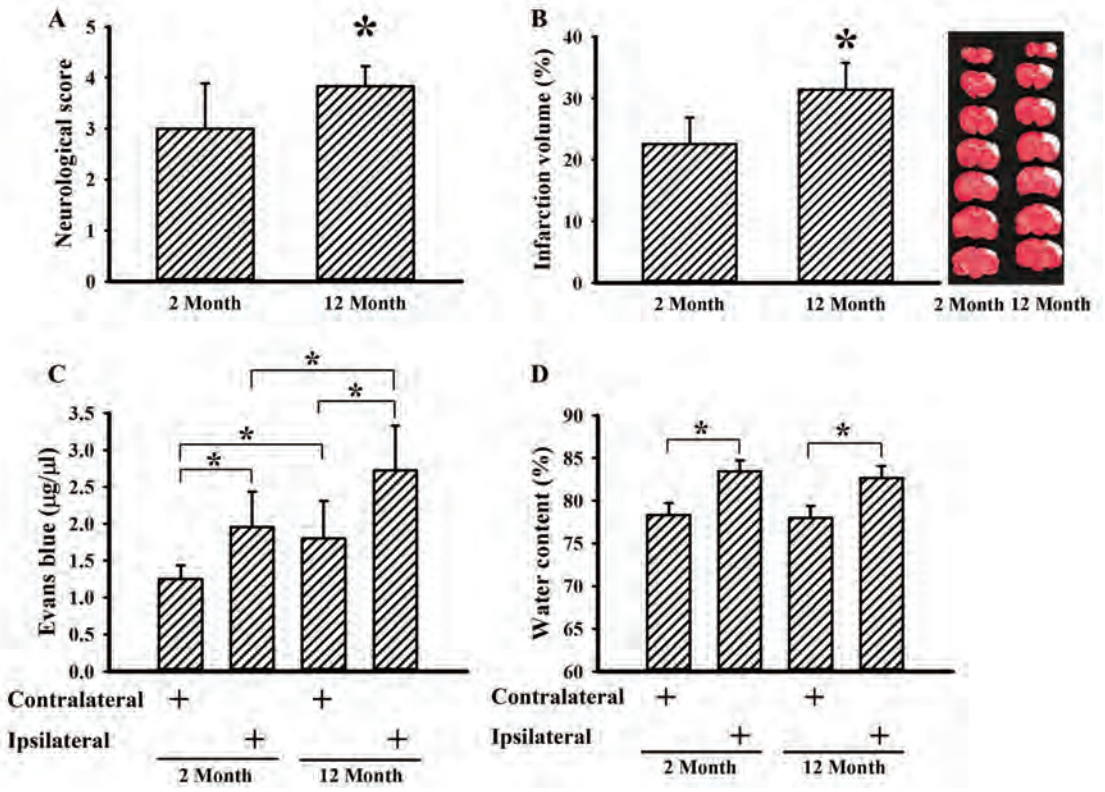


**Fig. 2** Middle-aged rats developed worse stroke outcomes. Rats aged 2 or 12 months were subjected to arterial occlusion for 1 day to induce permanent cerebral ischemia. Neurological deficit was evaluated using a modified six-point neurological deficit severity scale (A). The average percentage of infarction volume in each ipsilateral hemisphere and their representative photographs are depicted (B). BBB integrity was determined by measuring Evans blue content in contralateral and ipsilateral cortical tissues (C). Brain water content was measured in the ipsilateral and contralateral cortexes (D). *$p < 0.05$ vs. 2 months, $n = 6$.

intracerebral macrophage/microglia infiltration and activation and that these changes are associated with cerebral ischemia injury.

## Discussion

Stroke is a disease of the elderly. Using a rat model of stoke, we found that middle-aged animals exhibited lipid and glucose metabolism dysregulation along with systemic inflammatory activation. Middle-aged rats were susceptible to brain injury due to cerebral ischemia and showed exacerbated post-ischemic dysregulation of glucose metabolism and inflammation compared with young rats. Our results demonstrated that age-related comorbidities, such as hyperglycemia, insulin resistance, and chronic inflammation, predispose middle-aged rats to worse outcomes following cerebral ischemia injury.

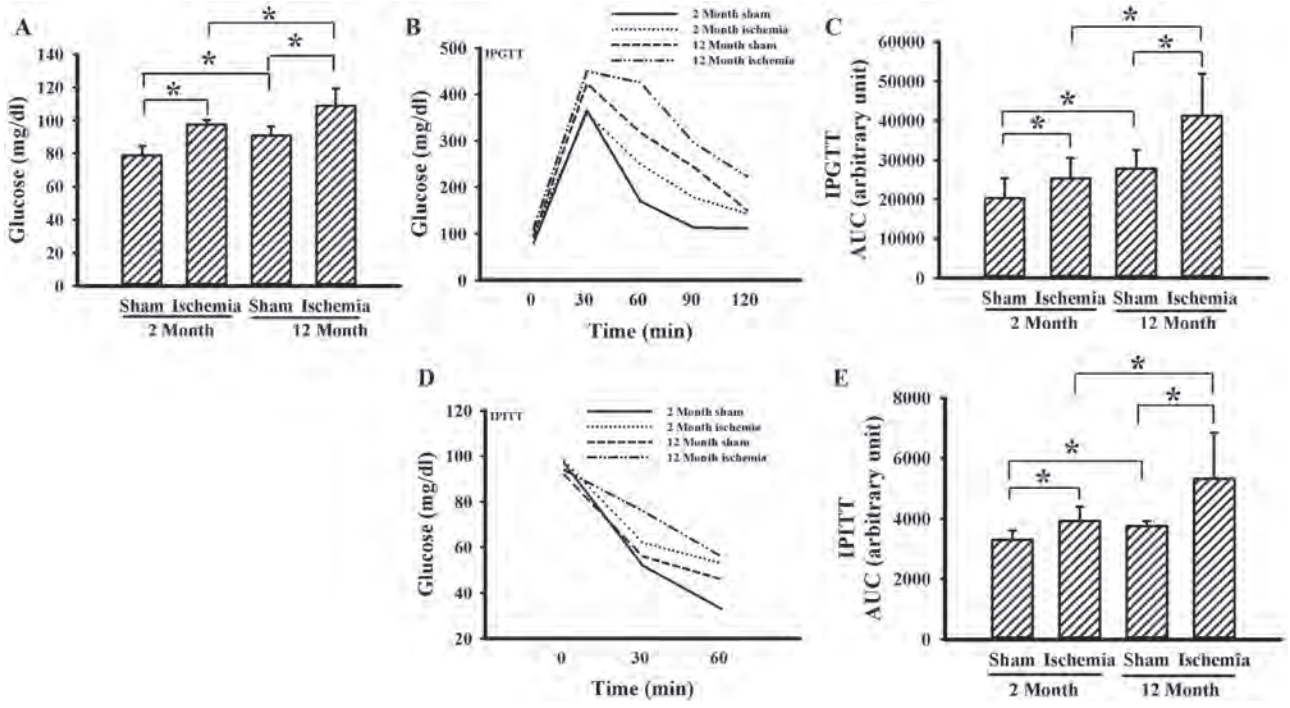


**Fig. 3** Middle-aged rats developed abnormal glucose metabolism. Rats aged 2 or 12 months underwent either sham operating or permanent cerebral ischemia surgery involving arterial occlusion for 1 day. After fasting overnight, the level of fasting blood glucose was measured (A). Blood glucose levels (B) and areas under the curve (AUC) (C) during an intraperitoneal glucose tolerance test were determined. Blood glucose levels (D) and AUC (E) during an intraperitoneal insulin tolerance test were determined. *p < 0.05, n = 6.

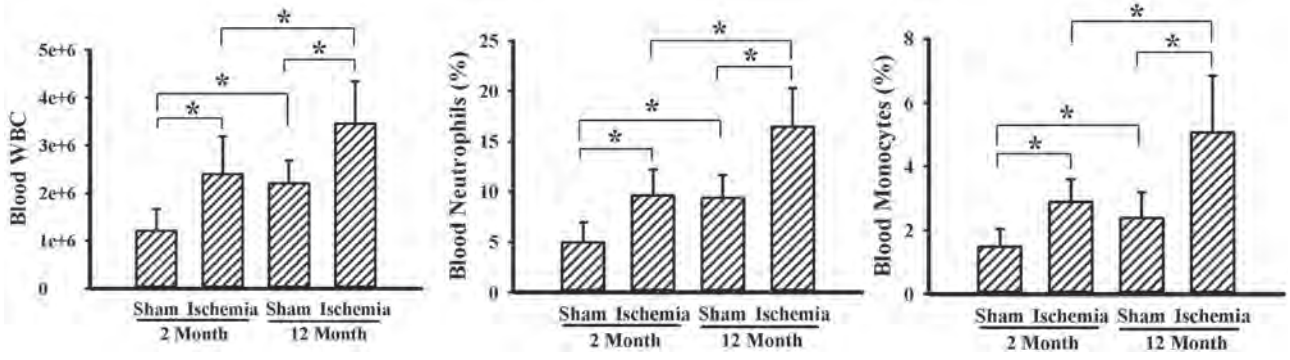


**Fig. 4** Middle-aged rats showed increased circulating leukocytes. Rats aged 2 or 12 months underwent either sham operation or permanent cerebral ischemia surgery involving arterial occlusion for 3 days. Blood samples were drawn from the left femoral artery and subjected to measurement of total leukocytes (WBCs), neutrophils, and monocytes. *p < 0.05, n = 6.

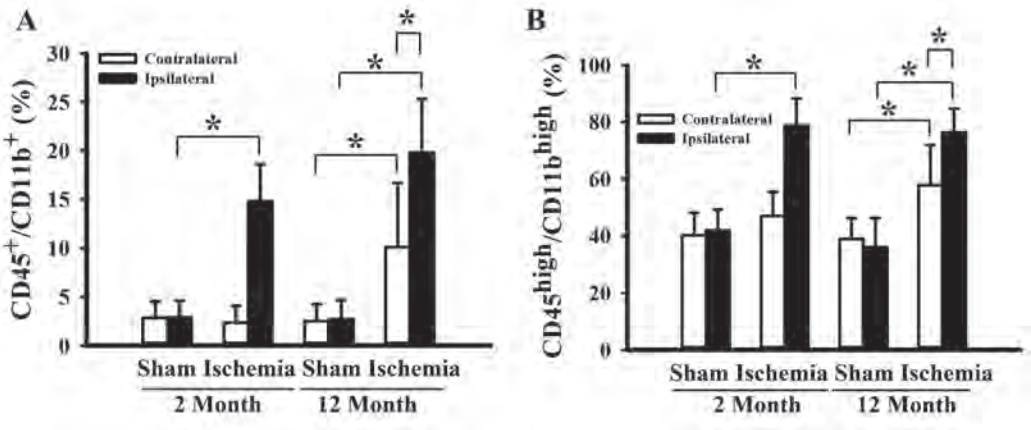


**Fig. 5** Middle-aged rats showed increased intracerebral macrophage/microglia infiltration and activation. Rats aged 2 or 12 months underwent either sham operation or permanent cerebral ischemia surgery involving arterial occlusion for 3 days. Leukocytes were isolated from contralateral and ipsilateral cortical tissues and subjected to flow cytometry analysis using antibodies recognizing CD5 and CD11b. Average percentages of $CD45^{+}/CD11b^{+}$ macrophages/microglia (A) and $CD45^{high}/CD11b^{high}$ activated macrophages/microglia (B) are depicted. *$p < 0.05$, $n = 6$.

Dysregulated lipid and glucose metabolism and inflammation are risk factors of post-ischemic brain injury[11, 13, 14]. Middle-aged rats exhibited pre-existing hyperlipidemia, hyperglycemia, hyperinsulinemia, insulin resistance, and systemic inflammation, and they developed more severe neurological deficit and brain infarction following cerebral ischemia than young rats. These data demonstrate a strong association of stroke injury with dysregulated lipid and glucose metabolism as well as inflammation.

The increased levels of CRP and circulating WBCs, neutrophils, and monocytes in the bloodstream reflected a pro-inflammatory condition in middle-aged rats. Similar studies have shown the concurrent presence of hyperglycemia, hyperinsulinemia, glucose intolerance, and insulin intolerance in middle-aged rats. The accompanying inflammatory responses and metabolic abnormalities in rats with stroke have also been reported. The initiation and maintenance of chronic inflammation are complicated processes involving multiple mechanisms. Hyperglycemia and insulin resistance represent alternative mechanisms underlying the induction of inflammation[15]. Thus, our findings suggest a close crosstalk between metabolic abnormalities and inflammatory responses in middle-aged rats with post-ischemic changes.

The exacerbated ischemic brain injury observed in middle-aged rats was evidenced by neurological deficit, brain infarction, and BBB disruption, although there was no brain edema. Why there was no apparent brain edema in these middle-aged stroke rats remains unclear. Our data revealed interesting findings regarding the evaluation of the BBB integrity. Similar to that in a previous study[11], cerebral ischemia disrupted the BBB integrity and enabled the extravasation of Evans blue into the ipsilateral cortical tissues of the young and middle-aged rats. Intriguingly, the BBB integrity in the contralateral cortical tissues of middle-aged rats was slightly impaired following cerebral ischemia compared with the sham groups. That is, the contralateral hemisphere maintained the appropriate BBB integrity under normal conditions; however, it was prone to disruption in the presence of physiological insults. Our previous study indicated critical roles of inflammatory cells and cytokines in the disruption of BBB integrity[16]. Here, we also noted elevated circulating leukocytes in middle-aged rats, with a parallel increase in intracerebral macrophage/microglia infiltration and activation in the contralateral cortical tissues of middle-aged rats after cerebral ischemia. As opposed to that in young rats, the lack of further elevation in intracerebral macrophage/microglia infiltration and activation in middle-aged rats might be due to the increase of these two factors in both the contralateral and ipsilateral hemispheres of middle-aged rats following cerebral ischemia. Based on relevant studies and our findings, we hypothesize that age-associated molecular and cellular dynamics render rat brains vulnerable to ischemic injury. The elevated systemic inflammatory status could play a key role, along with ischemic insults, in disrupting BBB integrity in the contralateral

hemisphere of middle-aged rats. However, further investigation is warranted to confirm this hypothesis.

In conclusion, the present study demonstrated that middle-aged rats showed more severe brain injury-related sequelae following cerebral ischemia than young rats. Prior to the induction of cerebral ischemia, middle-aged rats exhibited with hyperlipidemia, hyperglycemia, hyperinsulinemia, insulin resistance, and chronic low-grade inflammation. These metabolic abnormalities rendered the older rats prone to worse sequelae following brain ischemia. In other words, post-ischemic brain injury further augmented the older rats' pre-existing metabolic abnormalities. These results in the rodent model of stroke shed light on the mechanisms of brain ischemia in young and middle-aged rats and could form the basis for further research on stroke in the elderly.

## Acknowledgments

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# 年輕及中年中風鼠缺血後腦部傷害的差異比較

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**摘要**

腦中風是老年人好發的疾病之一。腦中風的發生率、失能、死亡率會隨著年齡增長而上升。年齡相關的共病症複雜，顯示老年人的腦中風神經傷害高風險及惡化是多因性的作用結果。為了進一步擴展老年腦中風傷害惡化相關研究，本研究探討年齡與共病因子的腦中風神經傷害相關性及找尋牽涉的惡化因子。相對於 2 月齡（年輕）雄性 Sprague-Dawley 品系大鼠，12 月齡鼠（中年），展現高血脂、高血糖、胰島素抗性、慢性低程度發炎的生理狀態。透過結紮雙側頸總動脈及右側中腦動脈的腦中風大鼠模式，實驗發現中年鼠的腦中風神經傷害嚴重度高於年輕鼠。中年鼠的代謝異常生理狀態促使腦中風後的神經傷害性增加。相對的，腦中風後的神經傷害也進一步惡化代謝異常。整體而言，我們的研究發現顯示，高血脂、高血糖、胰島素抗性、慢性發炎等年齡相關共病，促使中年大鼠的腦中風神經傷害性增加。以年輕及中年鼠腦中風模式所闡述的危害因子及神經傷害機制，將奠定後續老年腦中風相關的研究基礎。

**關鍵詞：**老年、發炎、代謝、中風

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Original Article

# Association of Vascular Endothelial Growth Factor Gene rs25648 Polymorphism and Risk of Bladder Cancer: A Meta-Analysis

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**Abstract**

***Background and Purpose*:** Vascular endothelial growth factor (VEGF) plays a crucial role in the angiogenesis of bladder cancer. The relationship between *VEGF* −7C/T (rs25648) polymorphism and risk of bladder cancer was studied extensively in recent years, albeit with ambiguous conclusions. Therefore, we conducted the present systematic review and meta-analysis to evaluate the association between *VEGF* −7C/T (rs25648) polymorphism and the risk of bladder cancer.

***Methods*:** We performed a comprehensive literature search in PubMed, Embase, and Google Scholar databases to identify eligible literature up to February 2019. Pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of the association. The stability of our analysis was evaluated using heterogeneity, sensitivity, subgroup, and publication bias analyses.

***Results*:** Three case-control studies comprising 1,645 patients with bladder cancer and 2,009 controls fulfilling the inclusion and exclusion criteria were selected for this meta-analysis. The pooled ORs for bladder cancer under an allele model (T vs. C) and a dominant model (T/T+C/T vs. C/C) were 1.31 and 1.26, respectively, with no inter-study heterogeneity. Under a recessive model (T/T vs. C/C+C/T), significantly increased bladder cancer risks of 2.34 and 2.44 were determined for the fixed effect and random effects models, respectively. Subgroup analysis revealed significantly increased bladder cancer risks in both Asian and Spanish populations.

***Conclusion*:** Our findings suggest that *VEGF* −7C/T (rs25648) polymorphism is significantly associated with bladder cancer. Nonetheless, further studies with a larger sample size are needed to clarify the effects of *VEGF* polymorphisms on bladder cancer.

**Key words:** Bladder Cancer, Meta-Analysis, Polymorphism, Vascular Endothelial Growth Factor

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## Introduction

Bladder cancer ranks ninth among common cancers and thirteenth in terms of deaths worldwide [1]. Taiwan witnessed approximately 2,265 new cases of and 894 deaths from bladder cancer in 2016 [2]. Previous studies have determined that environmental

factors, such as tobacco smoking and occupational exposure to aromatic amines and polycyclic aromatic hydrocarbons, preventive strategies for bladder cancer. Increasing evidence suggests a significant influence of genetic predisposition on the incidence of bladder cancer [3].

Among the various hallmarks of tumorigenesis, the formation of new blood vessels from pre-existing ones, known as angiogenesis, is critical in the development and prognosis of various cancers, including bladder cancer [4]. The vascular endothelial growth factor (VEGF) is a central regulator in the process of angiogenesis [5]. Notably, VEGF overexpression can be detected in various cancers, including bladder cancer [6]. An in vivo study revealed that anti-VEGF antibodies inhibited the growth of tumors in mice [7]. Such relevant results have motivated the treatment of advanced bladder cancer with bevacizumab (a VEGF antibody) in combination with chemotherapy during late-stage clinical trials [8].

*VEGF* gene is located on the chromosome 6p21.3 region and consists of eight exons [9]. Several single nucleotide polymorphisms (SNPs) located in the *VEGF* gene have been identified to be associated with the susceptibility and prognosis of some cancers. The −7C/T (rs25648) polymorphism is located in the 5′ untranslated region (5′ UTR) of the *VEGF* gene and plays a role in the carcinogenesis. A previous study reported that the T/T genotype of −7C/T polymorphism was found to be associated with higher levels of VEGF expression [10]. Those individuals who carried the *VEGF* −7C/T (rs25648) have been observed to have increased VEGF mRNA levels in patients with colorectal or urogenital malignancies [11,12]. Even though the *VEGF* −7C/T (rs25648) polymorphism was extensively studied in recent years, it produced ambiguous conclusions. Therefore, we conducted this systematic review and meta-analysis to investigate the role of *VEGF* −7C/T (rs25648) polymorphism in bladder cancer.

## Methods

### Eligible study selection

Eligible studies regarding the association of *VEGF* −7C/T polymorphism and bladder cancer were retrieved from a comprehensive literature search, without any language restriction, on PubMed, Embase, and Google Scholar up to February 2019. The following terms and their combinations were searched in the title and abstract: "vascular endothelial growth factor" or "*VEGF*"; "polymorphism" or "genotype"; "rs25648" or "−7C/T" or "promoter"; and "bladder cancer" or "urothelial carcinoma." The reference lists of included studies and review articles were manually searched to find relevant studies. The comprehensive review of literature research was conducted by two independent authors (CH Lien and WL Wu). Disagreements concerning certain literature reviews were resolved by discussion, and then two independent senior investigators (YS Chen and YH Wang) were consulted to make a final decision.

### Inclusion and exclusion criteria

The inclusion criteria comprised the following: (1) human subjects study; (2) studies that evaluated the association between *VEGF* −7C/T (rs25648) polymorphism and bladder cancer; (3) case-control study; (4) useful genotype data of cases and controls should be available. This study excluded reviews, comments, and animal studies.

Based on the inclusion criteria, we finally identified a total of three eligible literature for the present meta-analysis (Figure 1).

### Data extraction

Data from the included studies were extracted independently by two authors (CH Lien and WL Wu). The following details were extracted: first author, publication year, country, ethnicity, sample size (the number of cases and controls), genotyping method, genotype frequency of *VEGF* −7C/T (rs25648) polymorphism, and estimation of Hardy-Weinberg equilibrium (HWE) in controls.

### Statistical analysis

For each study, we first inspected the genotype frequencies in the controls under the HWE, which was assessed using the goodness-of-fit Chi-square test. The strength of the association between *VEGF* −7C/T (rs25648) polymorphism and bladder cancer was estimated using odds ratio (OR), with corresponding 95% confidence interval (CI). Because the exact underlying mode of inheritance of the *VEGF* allele in bladder cancer outcome is unknown, both the dominant (T/T +C/T vs. C/C) and recessive (T/T vs. C/C+C/T) models were used to investigate the association.

Inter-study heterogeneity was examined using

Cochran's Q test, $I^2$ statistic, and through the visual inspection of forest plots. If there was significant evidence to suggest inter-study heterogeneity, the random effects model was applied to calculate the pooled OR; otherwise, the fixed effects model was used. In addition, a subgroup analysis based on ethnicity was conducted to assess the possible cause of heterogeneity.

A sensitivity analysis was conducted to assess the influence of each study on the estimated pooled effect by removing each study from the data set. Publication bias was examined using the Begg's test and Egger's test. All statistical analyses were performed using the Review Manager, Version 5.3 (The Cochrane Collaboration, Oxford, England) and Comprehensive Meta-Analysis Software, Version 3.3.070. The present meta-analysis was conducted according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [13].

## Results

### Characteristics of included studies

Overall, 415 records relevant to the search terms were initially identified, and then three eligible literature were included for this meta-analysis (Fig. 1). The

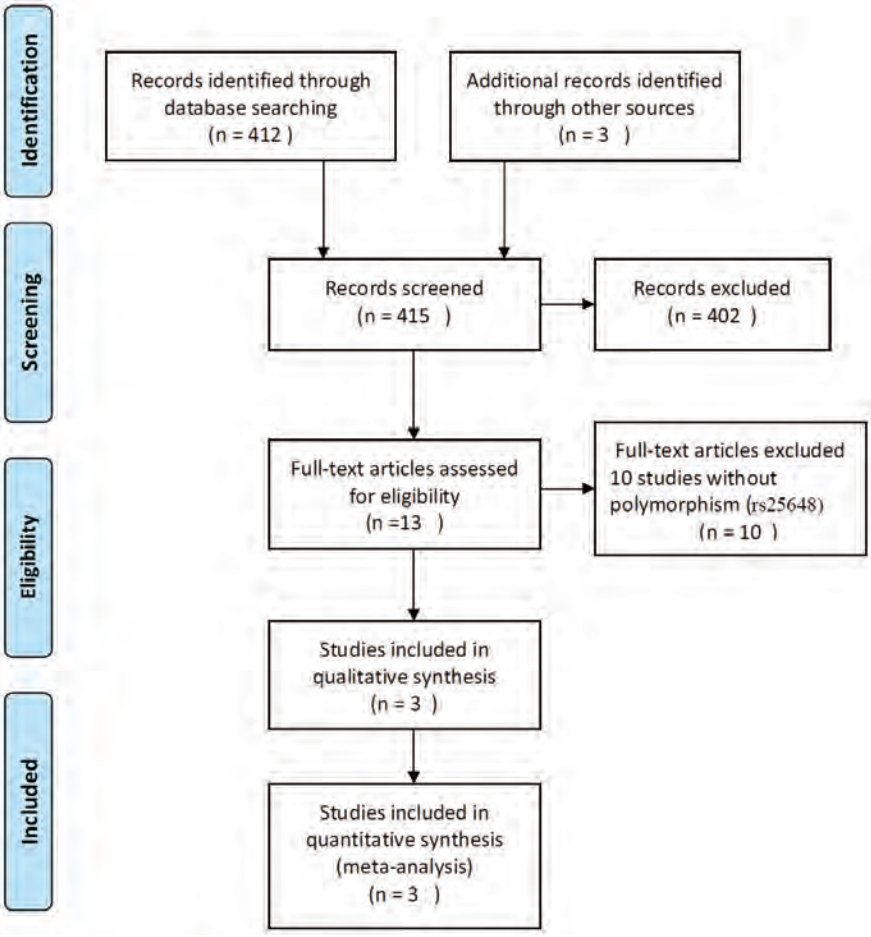



**Fig. 1** Flow diagram of study selection.

**Table 1.** Basic characteristics of studies included in the present meta-analysis

| First author | Year | Country | Ethnicity | Sample size (case / control) | Genotyping method* |
|---|---|---|---|---|---|
| Garcı´a-Closas[14] | 2007 | Spanish | European | 1085 / 1031 | A GoldenGate assay |
| Jaiswal[15] | 2013 | India | Indian | 200 / 250 | ARMS-PCR |
| Fu[16] | 2017 | China | Asian | 360 / 728 | PCR-RFLP |

*PCR-RFLP: Polymerase chain reactionrestriction fragment length polymorphism; ARMS: Amplification-refractory mutation system.

basic characteristics of the included studies are presented in Table 1. These three included studies were conducted in three countries of Europe and Asia, with the publication years ranging from 2007 to 2017. The genotype frequencies of *VEGF* −7C/T polymorphism among controls and cases are presented in Table 2. Overall, 1,645 patients with bladder cancer and 2,009 controls were included. All genotype frequencies in the control population among the included studies were in agreement with those predicted under HWE ($P > 0.05$).

### Main findings for VEGF rs25648 polymorphism and sensitivity analysis

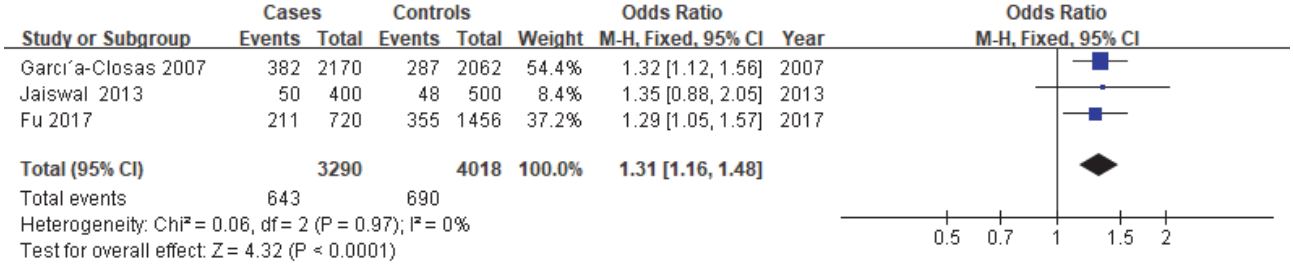
The pooled OR for bladder cancer risk in subjects with the T allele compared with those having the C allele was 1.31 (95% CI =1.16–1.48, $P < 0.001$), with no inter- study heterogeneity ($I^2 = 0\%$) (Fig. 2-1). In the dominant model, the pooled OR for bladder cancer risk in subjects with the T/T+C/T genotypes compared with the C/C genotype was 1.26 (95% CI = 1.09–1.45, $P = 0.002$), with no significant inter-study heterogeneity ($I^2 = 0\%$) (Fig. 2-2).

However, a significant level of heterogeneity ($I^2 = 73\%$, $P = 0.03$) was observed for the recessive model (Fig. 2-3). Under the recessive model, compared with subjects carrying the C/C+C/T genotypes, those with the T/T genotype had significant bladder cancer risks under a fixed effect model (OR = 2.34, 95% CI = 1.64–3.34, $P < 0.001$) as well as the random effect model (OR = 2.44, 95% CI = 1.03–5.78, $P = 0.04$).

Furthermore, to identify the potential causes of heterogeneity and assess the consistency of the present meta-analysis, we performed a sensitivity analysis by omitting each study one by one. After excluding the study conducted by Garcı´a-Closas et al. (2007) [14], the heterogeneity was reduced ($I^2 = 0\%$), indicating that the heterogeneity could have resulted

**Table 2.** The distribution of *VEGF* -7C/T (rs25648) polymorphism among included literatures

| First author | Year | Genotype frequencies for cases | | | | Genotype frequencies for controls | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | C/C | C/T | T/T | T allele (%) | C/C | C/T | T/T | T allele (%) |
| Garcı´a-Closas[14] | 2007 | 746 | 296 | 43 | 17.6 | 752 | 271 | 8 | 13.9 |
| Jaiswal[15] | 2013 | 155 | 40 | 5 | 12.5 | 206 | 40 | 4 | 9.6 |
| Fu[16] | 2017 | 186 | 137 | 37 | 29.3 | 421 | 259 | 48 | 24.4 |



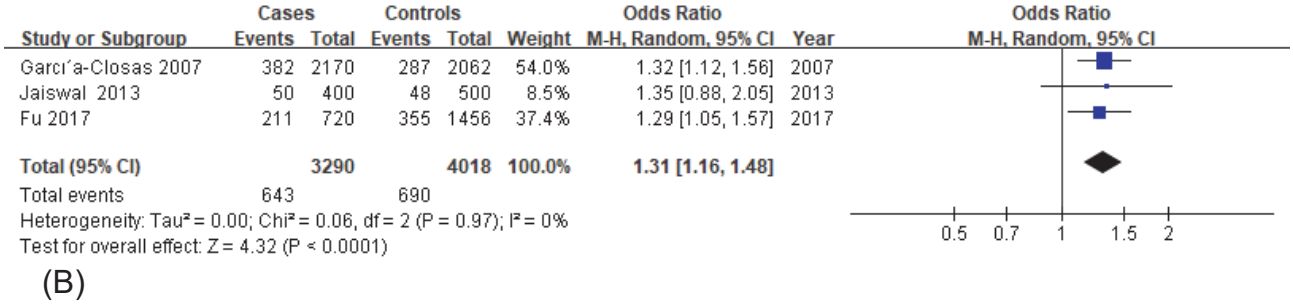
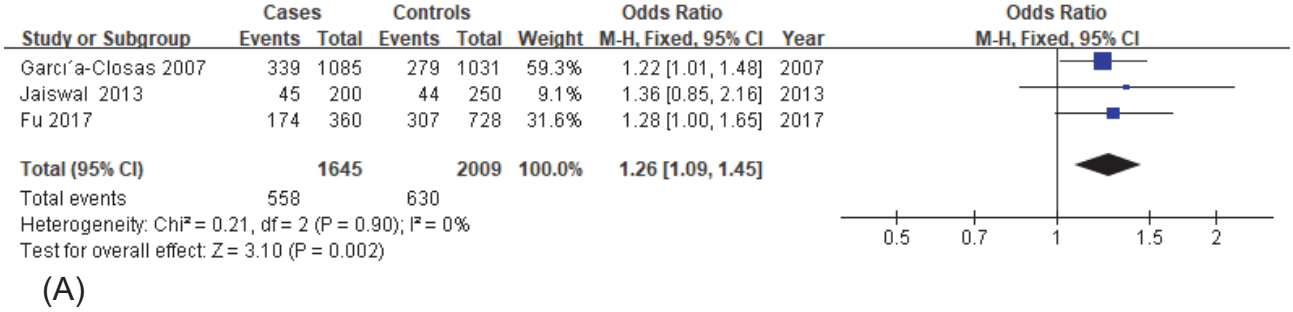




**Fig. 2-1** Allele comparison: C vs. T. (A) fixed-effect model; (B) random-effect model.

from the ethnicity. Furthermore, the pooled OR reduced to 1.62 (95% CI = 1.06–2.48; *P* = 0.027) under the recessive model, because this excluded study accounted for approximately 57.9% of the overall individuals included in this analysis. The remaining studies did not exhibit significant influence over the



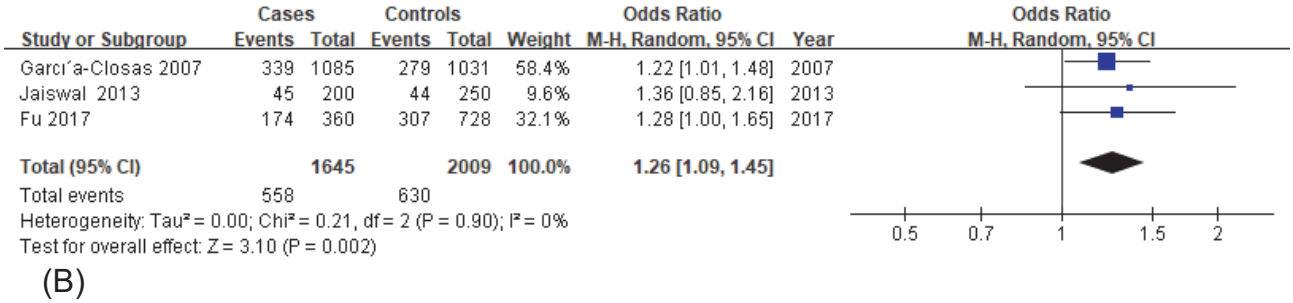
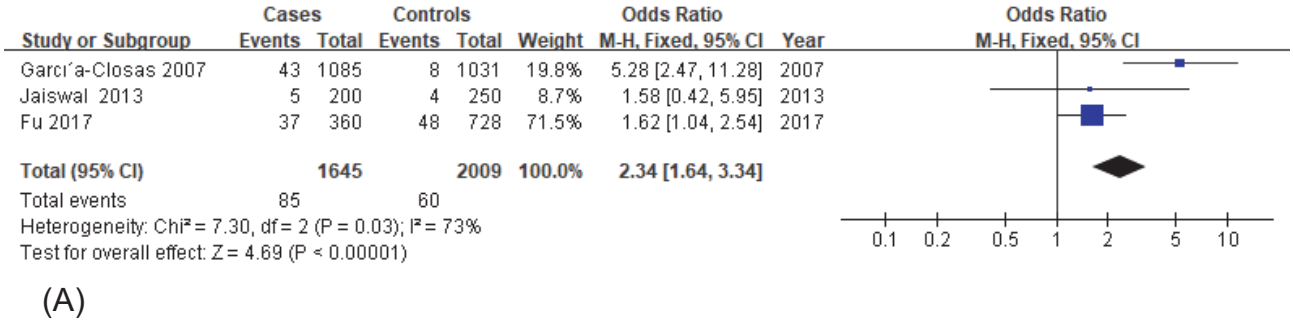




**Fig. 2-2** Dominant model. (A) fixed-effect model; (B) random-effect model.



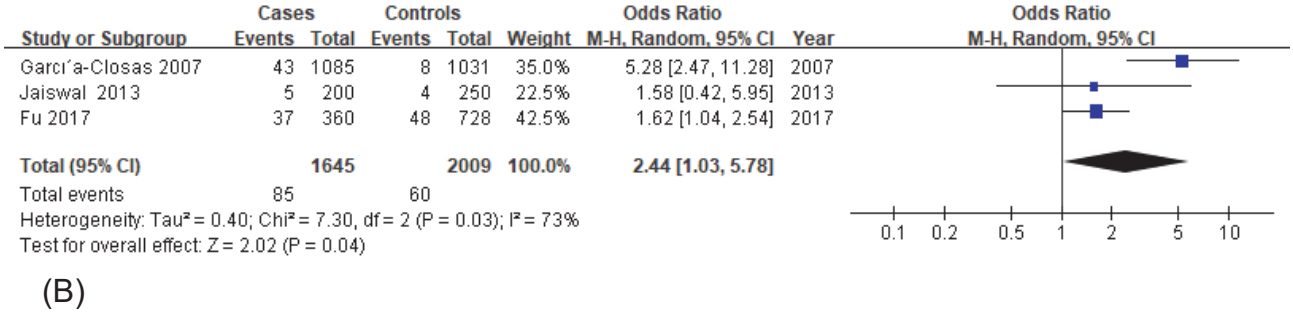




**Fig. 2-3** Recessive model. (A) fixed-effect model; (B) random-effect model.

**Table 3.** Subgroups analysis for *VEGF* -7C/T (rs25648) polymorphism and bladder cancer risk

| | No. | Model | T/T+C/T versus C/C (Dominant model) | | T/T versus C/C+C/T (Recessive model) | |
|---|---|---|---|---|---|---|
| | | | OR (95%CI) | P-value | OR (95%CI) | P-value |
| Total | 3 | Fixed | 1.26 (1.09-1.45) | 0.002 | 2.15 (1.48-3.11) | <0.001 |
| | | Random | 1.26 (1.09-1.45) | 0.002 | 2.44 (1.04-5.70) | 0.040 |
| Ethnicity | | | | | | |
| Asian | 2 | Fixed | 1.30 (1.04-1.62) | 0.021 | 1.62 (1.06-2.48) | 0.027 |
| | | Random | 1.30 (1.04-1.62) | 0.021 | 1.62 (1.06-2.48) | 0.027 |
| European | 1 | Fixed | 1.23 (1.02-1.48) | 0.035 | 5.28 (2.47-11.28) | <0.001 |
| | | Random | 1.23 (1.02-1.48) | 0.035 | 5.28 (2.47-11.28) | <0.001 |

pooled effect estimate under the allelic, dominant, or recessive models.

**Subgroup analysis**

Among the three included literature, only one study with the European population was conducted in Spain [14]. The other two eligible studies with the Asian population were conducted in India and China, respectively [15,16]. Table 3 presents the subgroup analysis based on ethnicity to estimate the effect of *VEGF* −7C/T (rs25648) polymorphism on bladder cancer development. In the dominant model, we observed that the findings of subgroup analysis remained similar to that of the principal analysis. The OR (95% CI) for the Asian population was 1.30 (1.04–1.62), and for the Spanish population was 1.23 (1.02–1.48). In the recessive model, significantly higher risks of 1.62 (1.06–2.48) and 5.28 (2.47–11.28) were determined for the Asian and Spanish populations, respectively.

**Publication bias**

Because the publication bias could affect the pooled findings of the present meta-analysis, we performed the funnel plot and Egger's test to evaluate the potential publication bias. No statistically significant evidence of publication bias was observed for the included literature in the present meta-analysis.

## Discussion

The major finding of this meta-analysis evidenced that *VEGF* −7C/T (rs25648) polymorphism is associated with the risk of bladder cancer. Results from our pooled analysis showed that individuals carrying T allele might be more susceptible to developing bladder cancer. In the subgroup analysis, significantly increased risks of bladder cancer were observed for both Asian and Spanish populations.

After a comprehensive literature search, only three eligible studies were found that were related to the association between *VEGF* −7C/T (rs25648) polymorphism and bladder cancer risk. *VEGF* −7C/T (rs25648) polymorphism is located within 5' UTR of the *VEGF* gene and is functionally crucial in angiogenesis; hence, we analyzed the *VEGF* −7C/T polymorphism and observed its significant association with bladder cancer. Earlier studies have reported that the variant allele of *VEGF* −7C/T polymorphism results in an increased level of VEGF mRNA in colorectal or urogenital cancers [11,12]. García-Closas et al. [14] reported that several polymorphisms located in the promoter region were associated with the deregulation of target genes, thereby providing an explanation that −7C/T polymorphism of *VEGF* gene affects the mRNA stability and expression of VEGF.

However, the study conducted by Jaiswal et al. [16] in India indicated that the *VEGF* −7C/T polymorphism might not be significantly associated with bladder cancer. Other studies reported that no significant association was observed between *VEGF* −7C/T polymorphism and cervical cancer or papillary thyroid carcinoma [17,18]. The inconclusive observations of these studies could be related to the ethnicity, lifestyle factors, and geographical distribution that may have played a significant role in the progression and risk of various cancers.

The present study observed evident heterogeneity among the studies concerning *VEGF* −7C/T polymorphism. Sensitivity analysis was conducted by omitting each study at a time and revealed that the heterogeneity declined and the pooled ORs reduced

after removal of the study by Garcı´a-Closas et al. [14]). This finding could probably be due to the variations in genetic frequency among different populations. Table 2 indicates that the T allele frequency (9.6%–24.4%) in the Asian population, especially the Han population, is higher than that in the European population (13.9%). Therefore, we performed a subgroup analysis based on ethnicity. Significantly increased risks were observed for both Asian and Spanish populations. Moreover, the $I^2$ statistic significantly decreased ($I^2$ = 0%), indicating that the ethnicity of the included studies is the primary cause of heterogeneity.

VEGF has been identified as a critical factor in angiogenesis required for tumor growth. Earlier studies have indicated a correlation between VEGF expression and the occurrence and progression of bladder cancer [19]). Zhang et al. [20] reported that VEGF expression level was significantly associated with the stage, grade, and lymph node metastasis of bladder cancer. A previous in vitro study has shown that variant allele of *VEGF* −7C/T polymorphism was associated with higher levels of VEGF mRNA [11]. It suggests that the *VEGF* polymorphisms may be potential biomarkers for the prognosis as well as therapeutic targets of bladder cancer. Recent studies conducted the haplotype analysis to explore the effects in the combinations of *VEGF* polymorphisms and revealed that some haplotypes of the *VEGF* gene were related to higher VEGF production in peripheral blood mononuclear cells and could be a significant risk factor for bladder cancer [21-23]. Therefore, additional large-scale studies with functional polymorphisms of the *VEGF* gene are needed for evaluating the association between *VEGF* polymorphisms and the efficacy of bladder cancer treatments.

Nonetheless, this meta-analysis had several potential limitations. First, the number of studies included in our meta-analysis was small. Second, gender and environmental exposures were not obtained from each study, which limited our further exploration of the combined effect of gene-gene or gene-environment interaction. Third, most of the cases and controls in our included studies belonged to the Asian and Spanish ethnicity. Therefore, the results of our pooled effect may not be extrapolated to other ethnicities. Last, the functional influence of *VEGF* −7C/T polymorphism on bladder cancer is still unknown.

In conclusion, the present meta-analysis suggests that *VEGF* −7C/T (rs25648) polymorphism is substantially linked to the development of bladder cancer. Moreover, *VEGF* −7C/T (rs25648) polymorphism was observed to be significantly related to the risk of bladder cancer in both the Asian and Spanish populations. Nevertheless, further population-based studies are warranted to detect the gene-gene and gene-environment interactions, as well as the association between the *VEGF* −7C/T (rs25648) polymorphism and bladder cancer susceptibility.

## Acknowledgements

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# 血管內皮生長因子 rs25648 基因多形性與膀胱癌的相關性：統合分析

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**摘要**

**背景及目的：**血管內皮生長因子在膀胱癌的血管新生過程中扮演重要的角色。近年來，血管內皮生長因子的基因多形性（例如：rs25648）與膀胱癌的關係雖逐漸受到關注，然而這些研究結果仍然不一致。因此，本研究利用系統性文獻回顧與統合分析的方法進一步釐清血管內皮生長因子的基因多形性（rs25648）與膀胱癌的關係。

**方法：**本研究利用關鍵詞及完整的蒐尋相關文獻資料庫包括 PubMed 、Embase 及 Google Scholar 找尋符合納入 / 排除條件的相關文獻納入分析。文獻搜尋期間從過去截至 2019 年 2 月。利用統合的危險對比值與百分之九十五信賴區間估計相關性的強度。透過異質性、敏感度、次族群與發表偏誤的分析評估本研究分析結果的穩定性。

**結果：**本研究最後採用 3 篇符合納入 / 排除條件的文獻，合計 1,645 名膀胱癌個案及 2,009 名對照組個案的資料被萃取並進行統合分析。研究結果顯示在對偶基因（T vs. C）與顯性模式（T/T+C/T vs. C/C）下，帶有“T”對偶基因及“T/T+C/T”基因型者分別有 1.31 及 1.26 倍顯著較高罹患膀胱癌的風險。在隱性模式（T/T vs. C/C+C/T）下，帶有“T/T”基因型者在固定效應模型及隨機效應模型下分別具有 2.34 及 2.44 倍顯著較高罹患膀胱癌的風險。從次族群分析的結果顯示亞洲與西班牙族群帶有血管內皮生長因子危險基因型者皆有顯著較高的膀胱癌風險。

**結論：**本研究結果發現血管內皮生長因子基因多型性（rs25648）與罹患膀胱癌的風險有顯著相關。未來仍然需要較大型的研究進一步釐清血管內皮生長因子基因多形性在膀胱癌的致癌機轉、治療及預後所扮演的角色。

**關鍵詞：**膀胱癌、統合分析、基因多形性、血管內皮生長因子

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Case Report

# Aggressive Giant Cell Tumor of Bone in Elderly Female: an Unusual Age and Misdiagnosed on MRI as a Metastasis

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**Abstract**

***Background and purpose:*** Giant cell tumor (GCT) of bone, a locally aggressive albeit benign neoplasm, accounts for 3%–8% of all primary bone tumors. Approximately 60%–70% of patients with GCTs of bone are 20–40 years of age, whereas GCTs are very rare in those above the age of 55 years. Although many case studies on GCTs have been published, extensive search of the literature reveals few reports of elderly patients with GCTs. One case series of ten elderly patients with GCT of bone has reported that there were no differences in the characteristics of GCTs including tumor location, radiographic features, and clinical course present the case of a 74-year-old female with aggressive GCT of the left femoral condyle with an extended lesion, which was misdiagnosed by imaging studies.

**Key words:** diagnosis, elderly, magnetic resonance imaging

## Introduction

Giant cell tumor (GCT) of bone, also known as osteoclastoma, is a locally invasive but usually benign tumor arising in the epiphysis of bones. GCT of bone, which accounts for 3%–8% of all bone tumors, usually affects young adults in their third to fourth decades of life. GCT is very rare in patients who are either too young or too old [1]. We herein report a rare case of aggressive GCT of the distal femur in a 74-year-old female, which was misdiagnosed as a metastasis on radiographic and magnetic resonance imaging (MRI).

## Case Report

A 74-year-old female presented to the orthopedics clinic with progressively increasing pain and swelling in the left knee over the last two years. There was no history of fever, loss of appetite, weight loss, or past evidence of tuberculosis. The swelling was located over the distal left femur and was accompanied with a hard, tender mass fixed to the bone. However, the overlying skin did not have any sinuses or scars. Hematological and biochemical test results were within the normal range.

X-ray revealed a large, expanded, osteolytic area of bony destruction extending from the epiphysis to the metaphysis and a supracondylar pathological fracture (Fig. 1). Metastasis from a tumor was considered as the diagnosis. Contrast-enhanced MRI of the knee revealed a multilobulated soft tissue mass with bony destruction in the metaphyseal and epiphyseal regions of the distal femoral, which was marginally extending to adjacent soft tissue and exhibiting posterior lateral invasion (Fig. 2). Computed tomography of the chest and abdomen did not show chest or abdominal tumors.

Tumor-wide excision was performed, and custom mega prosthetic arthroplasty of the knee was selected due to the large defect (Fig. 3a–3b). *En bloc* tumor excision was performed, and tumor-free margins were confirmed by pathology. Histopathologically, scattered histiocytes and diffusely

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distributed multinucleated foreign-body giant cells were noted (Fig. 4a–4b). No malignant changes were observed. The definitive diagnosis was grade 2 GCT.

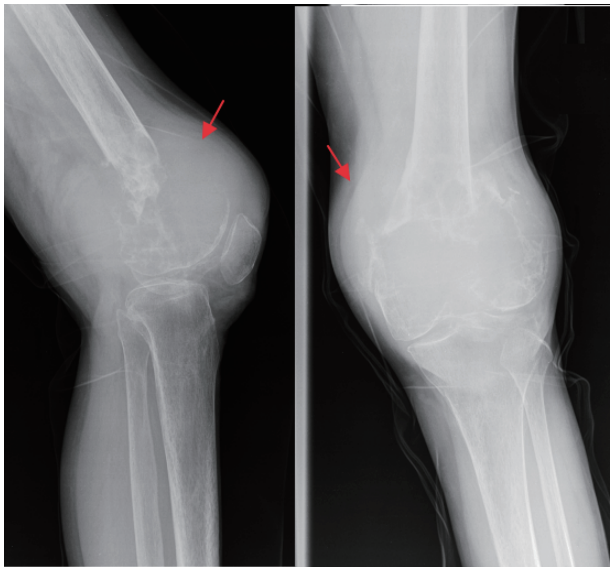


Fig. 1

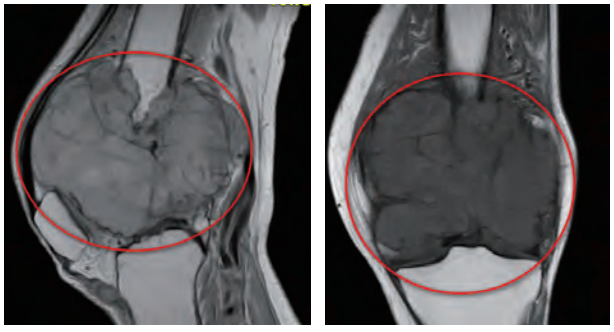
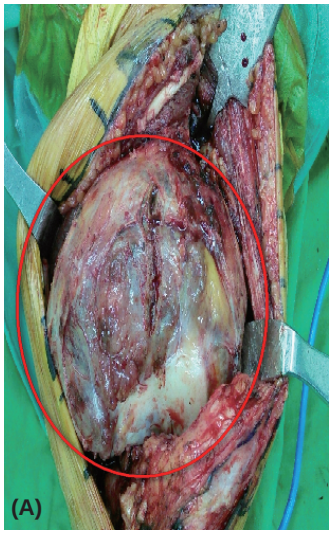


Fig. 2



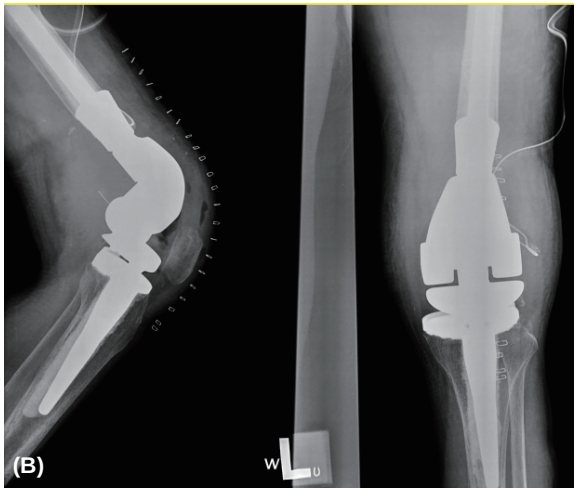




Fig. 3

## Discussion

GCT of bone usually affects young adults, and 60%–70% of the patients are in the age group of

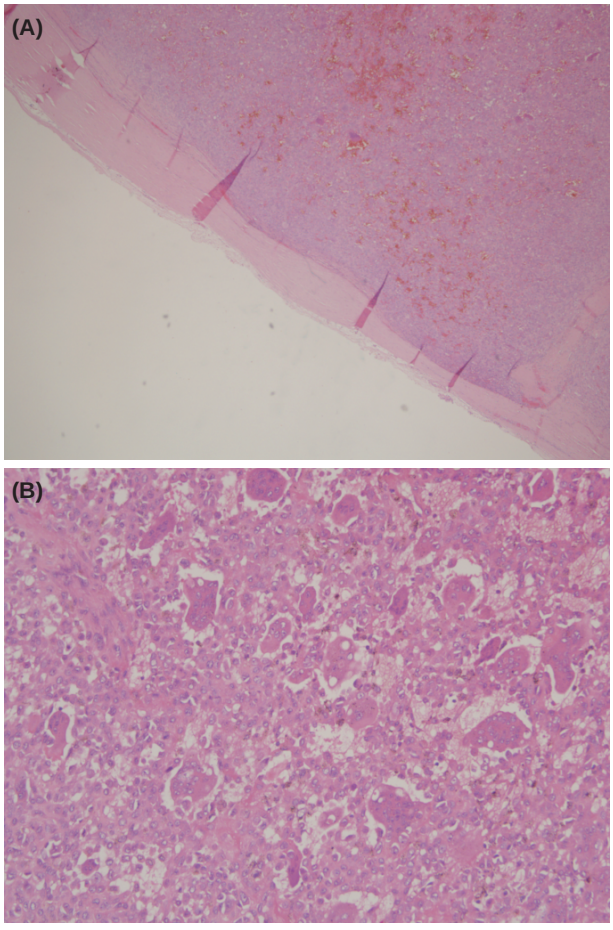



Fig. 4

20–40 years. Patients older than 55 years very rarely develop GCT [1]. Our literature review revealed only one case series by McCarthy *et al.* that focused on the GCT of bone in ten elderly patients ranging from 62 to 78 years of age [2]. The authors concluded that the GCT characteristics in elderly patients, including tumor location, radiographic features, and clinical course, were similar to those observed in younger patients.

The differentials of aggressive GCT are quite extensive. However, considering the older age of the patients, the differential diagnosis can be narrowed down to metastatic carcinoma, plasmacytoma, hemophilic pseudotumor, telangiectatic osteosarcoma, fibrosarcoma, and chondrosarcoma. The most common bone tumor in older patients is metastatic carcinoma. One factor that further complicates the differentiation of GCT from other metastatic carcinomas is that some carcinoma variants of the kidney, breast, and lung are rich in osteoclast-like giant cells [3, 4]. The possibility of a giant cell-rich osteosarcoma variant can be excluded based on the absence of malignant osteoid production by malignant tumor cells. Moreover, a uniform distribution of osteoclast-like giant cells, as observed in the present case, is not a feature of osteosarcoma. MRI is considered as the best imaging modality for GCT due to superior contrast resolution and multiplanar imaging capabilities that allow accurate tumor delineation. MRI is useful in determining the extraosseous extent and articular surface involvement. However, subtle cortical destruction can be better demonstrated by computed tomography. GCT exhibits low intensity on T1-weighted images and heterogeneous high intensity on T2-weighted images. Therefore, intramedullary tumors are best detected by T1-weighted imaging whereas their extraosseous portions are best appreciated on T2-wieghted images [5].

The use of a mega prosthesis has become the method of choice after bone tumor resection in the knee [6]. This approach is the preferred modality for the management of malignant bone tumors of lower limbs [7]. Custom mega prosthesis has been demonstrated to be a simple, technically superior method of replacing the lost bone segment in benign aggressive lesions with pathological fractures and in cases where disease progression has resulted in a clinical situation that prevents skeletal reconstruction after intralesional curettage [7, 8]. The advantages of custom mega prosthetic arthroplasty are cost-effectiveness, early resumption of knee function with unassisted ambulation, and lowest recurrence rates. The potential complications include flap necrosis, secondary infection, aseptic loosening fracture, and breakage [9, 10].

In summary, we herein presented the rare case of GCT in a patient in an unusual age group. The tumor was in the distal femur, a common location, exhibited a progressive appearance, and was misdiagnosed as a metastasis by MRI. The current case highlights that the possibility of GCT, albeit very unusual in the elderly, should be considered after taking the classical radiological findings into account and excluding other potential etiologies more common in this age group.

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# 侵襲性骨巨細胞瘤一個年長女性個案報告：少見發生年紀及核磁共振影像學誤診斷為腫瘤轉移

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受文日期：民國 107 年 01 月 11 日；接受刊載：民國 107 年 03 月 05 日

**摘要**

**研究背景與目的：**骨巨細胞瘤的盛行率大約占所有骨腫瘤的 3-8%，是一種良性局部侵襲性腫瘤，大約 60-70% 的病患年齡在 20-40 歲之間，病患年齡超過 55 歲以上者非常罕見，雖然目前已有許多骨巨細胞瘤的病例報告及相關研究，但是少文獻是在探討高齡病患的部分。

**方法：**我們找到有一篇關於骨巨細胞瘤在老年人的病例系列，來跟我們的個案來比較。

**結果及討論：**在年紀大及年輕的個案，骨巨細胞瘤在位置、影像檢查或臨床表現上並沒有很大差異，而我們提出一個個案的報告，是關於一個年長女性，在遠端股骨有個膨大的病灶（侵襲性的骨巨細胞瘤），影像學上被誤診的個案。

**關鍵詞：**骨巨細胞瘤、老年人、核磁共振診斷

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Case Report

# Unknown Origin Metastatic Malignant Melanoma of Omentum: A Case Report

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**Abstract**

Metastatic malignant melanoma of the greater omentum is very rare. Only one case of metastatic melanoma of the uterus with peritoneal seeding has been published. We present a case of metastatic malignant melanoma of the greater omentum without known origin in a 58-year-old male with a history of cerebral atherosclerosis, hypertension, and type 2 diabetes mellitus. He was admitted to our emergency department for acute right upper quadrant dull pain that had persisted for hours. A 6.5-cm mesenteric mass in the right upper abdomen was revealed by an abdominal computed tomography (CT) scan. A laparoscopic mass resection was performed. Metastatic malignant melanoma of the greater omentum was determined by histopathologic examination. Immunohistochemical analysis revealed that the melanoma was comprised of large neoplastic melanocytes with pleomorphism and melanin pigment deposition. The tumor cells were positive for the HMB45 and S100 proteins. The primary site may have originated from a skin lesion of the right big toe. An incisional biopsy was performed, and the histopathologic diagnosis was a compound nevus. The metastatic malignant melanoma was complicated with carcinomatosis two months later, and the patient died four days later due to multiple organ failure.

**Key words:** Greater omentum metastatic malignant melanoma, peritoneum seeding

## Introduction

Melanomas are usually recognized at an early stage when survival is high and surgery is often the only necessary treatment. Melanomas arise from the transformation of melanocytes, which are of neural crest origin. Most melanocytes reside in the basal layer of the epidermis or within benign common nevi. Malignant melanoma is an aggressive and metastatic cancer that originates from melanocytes. It occurs at various sites, most often in the skin and much less frequently in the choroid layers of the eyes, oral cavity, nasal mucosa, leptomeninges, pharynx, esophagus, bronchous, and vaginal and anorectal mucosa.[1] Common metastatic sites of malignant melanomas are the lungs, liver, lymph nodes, brain and meninges, bones, and gastrointestinal tract. Less frequently, malignant melanomas metastasize to the scalp, dura, eye, bile duct, duodenum, uterine cervix, vagina, rectum, anus, and peripheral nerves.[2] However, peritoneal seeding of a malignant melanoma is extremely rare. Treatment options for metastatic melanoma include molecularly targeted therapy based on the presence of predictive mutations, immunotherapy, cytotoxic chemotherapy, surgical resection of isolated metastases, and palliative care. The treatment of patients with metastatic melanoma depends on multiple factors, including the overall condition and age of the patient, the sites and number of metastases, and the patient's wishes for treatment.

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## Case report

A 58-year-old male with a history of cerebral atherosclerosis, hypertension, and type 2 diabetes mellitus for more than 10 years under the control of oral hypoglycemic agents (OHA) was admitted to our emergency department because of acute right upper quadrant dull pain that had persisted for hours. A 6.5-cm mesenteric mass in the right upper abdomen was revealed by an abdominal computed tomography (CT) scan. An adjacent mesenteric stranding edema and a small amount of hemoperitoneum in the pelvic cavity was noted (Fig. 1). A laparoscopic mass resection was performed (Fig. 2). However, metastatic malignant melanoma of the greater omentum was diagnosed by histopathologic examination. The tumor was 7.5 x 5.8 cm in size, with evident necrosis and hemorrhage. No other lesions were found during the operation. Immunohistochemical analysis revealed that it was a melanoma comprised of large neoplastic melanocytes with pleomorphism and melanin pigment deposition. The tumor cells were positive for the HMB45 and S100 proteins (Fig. 3).

The primary site may have been the patient's right big toe (Fig. 4). We performed an incisional biopsy, and the pathologic diagnosis was only a compound nevus (Fig. 5).

The metastatic malignant melanoma recurred two months later. An abdominal CT revealed recurrent metastatic melanoma with a diameter of more than 12 cm in the right lower abdomen, carcinomatosis abdominalis with thickening mesenteries, diffuse wall thickening of the bowels, and massive ascites (Fig. 6-1). Splenic metastasis was also suspected (Fig. 6-2). A staging laparoscopy was performed. Recurrent metastatic melanoma with carcinomatosis and

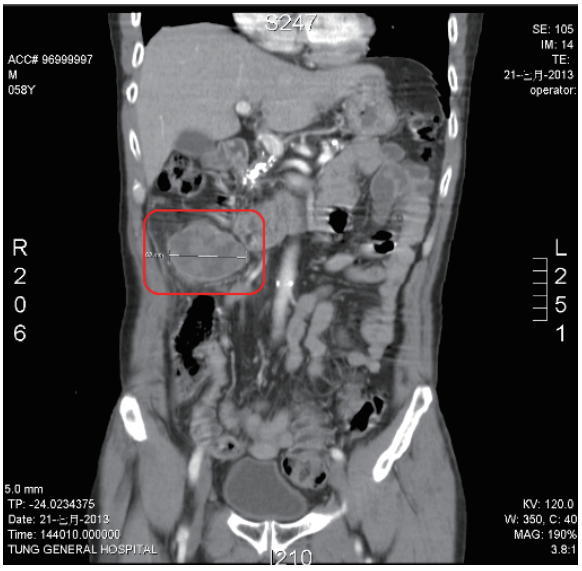


**Fig. 1** Abdominal CT scan revealed a 6.5-cm mass in the right upper abdomen.

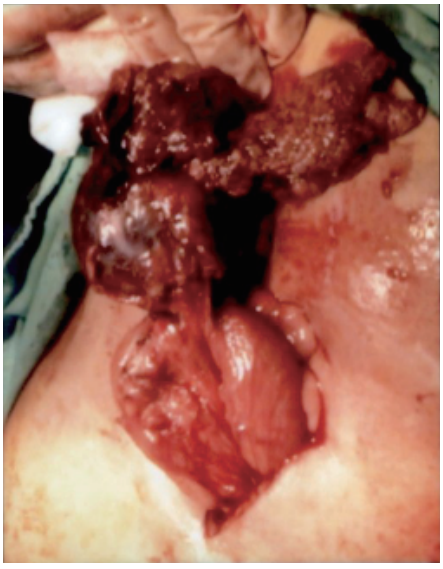

**Fig. 2** One greater omentum mass was found during laparoscopic surgery.

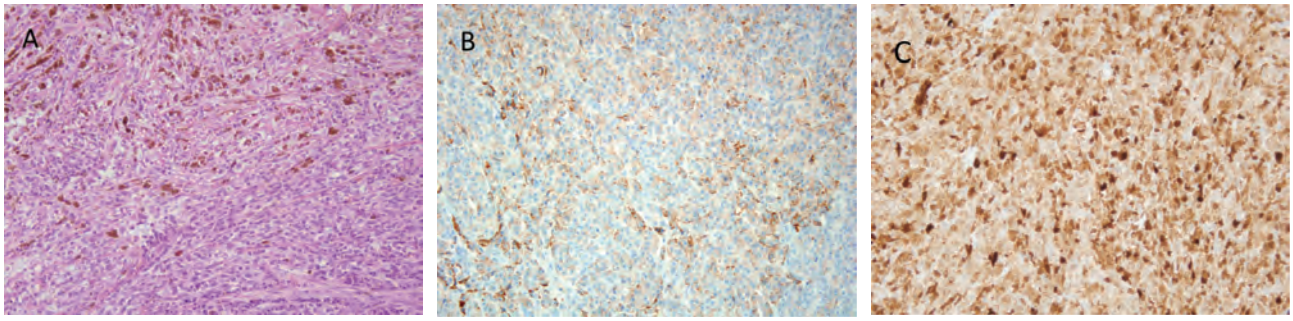


**Fig. 3** (A) Microscopic image of large neoplastic melanocytes (black arrow) with pleomorphism and melanin pigment deposition (H&E stain x 200). (B) The tumor cells are positive for HMB45 (IHC stain x 200). (C) Focal positive for the S100 protein (IHC stain x 200).

massive serosanguinous ascites of approximately 5400 ml were found during the operation (Fig. 7). This patient died four days later due to multiple organ failure.

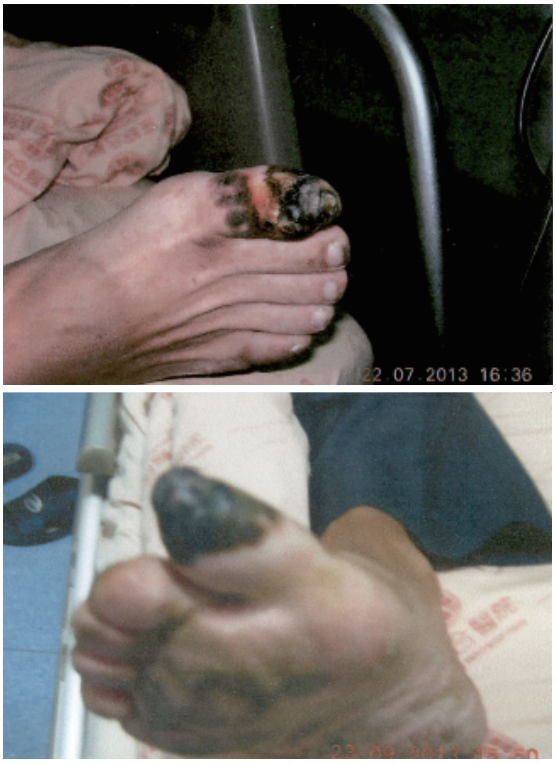


**Fig. 4** Down big toe skin lesion.

## Discussion

The worldwide incidence and mortality rates of malignant melanoma have been constantly increasing over the past 50 years in fair-skinned populations. Melanoma is the fourth most frequent type of cancer in Switzerland. Its incidence in Switzerland is one of the highest in Europe (24.6 per 100,000 population). [3] Approximately 20% of malignant melanomas will metastasize, whether by the hematogenic or

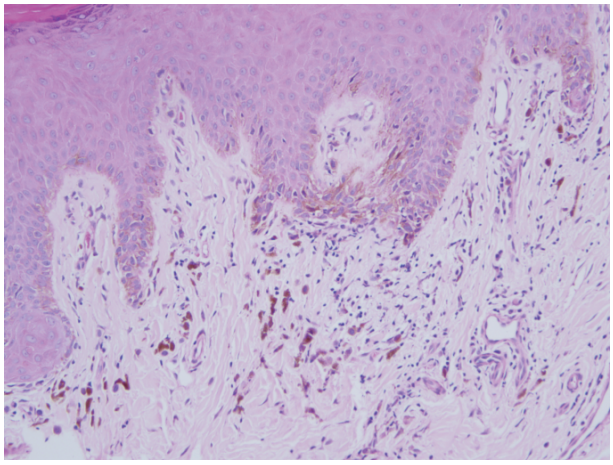

**Fig. 5** Microscopic view of sections of the compound nevus with a few small nevus cells and melanin pigment deposition in the basal of the epidermis and dermis area. No cellular atypia or mitosis is noted. No evidence of malignancy is present (H&E stain x 100).

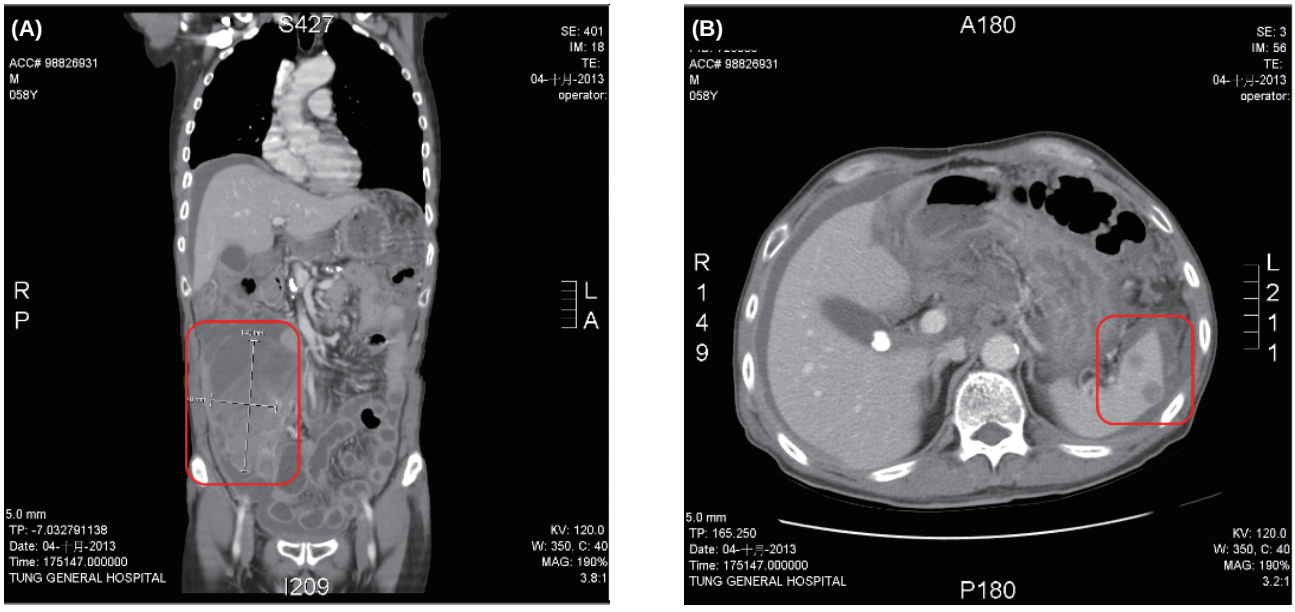


**Fig. 6** (A) Recurrent metastatic melanoma with a diameter of more than 12 cm in the right lower abdomen, carcinomatosis abdominalis with thickening mesenteries, diffuse wall thickening of the bowels, and massive ascites were noted two months later. (B) A suspected splenic metastasis was also found in the abdominal CT scan.

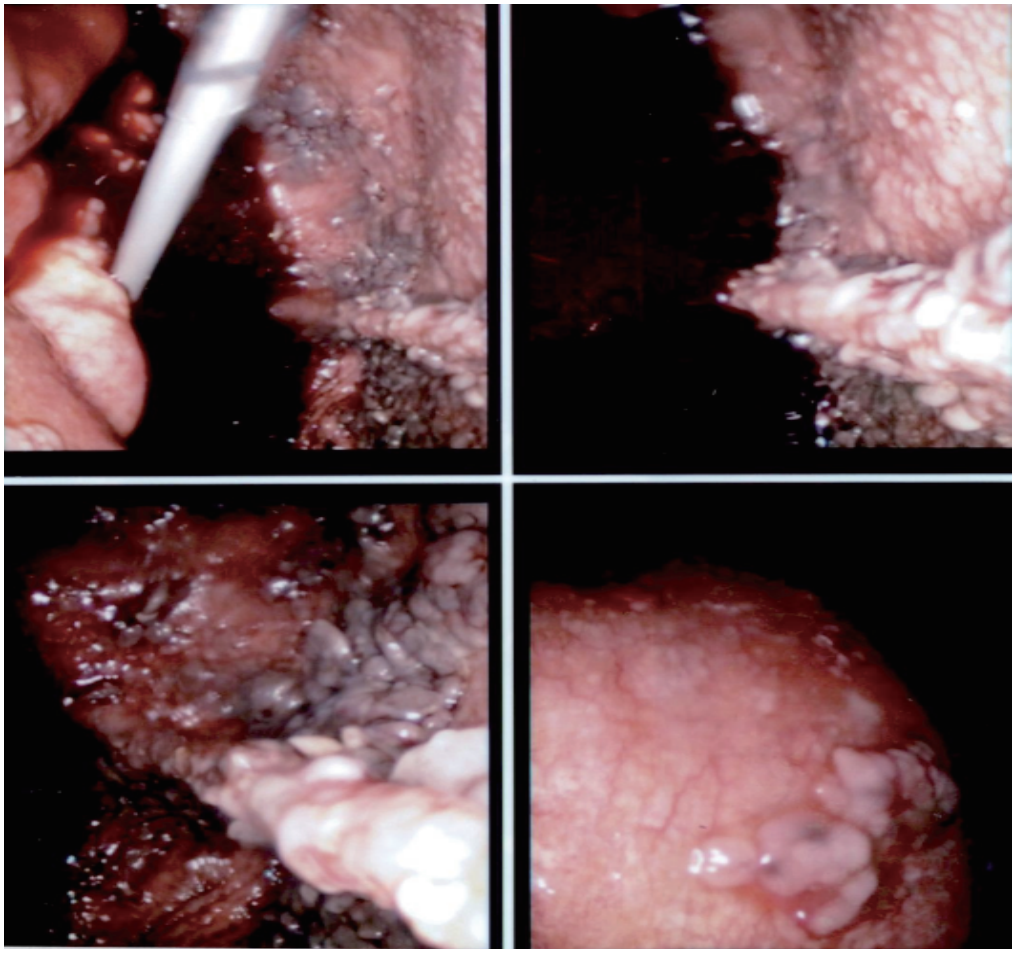

**Fig. 7** A recurrent metastatic melanoma with carcinomatosis was found during the staging laparoscopy surgery.

lymphatic route. Metastatic melanoma is usually found in the brain, bone, lungs, liver, and tissue under the skin and lymph nodes. The primary site is usually the skin because of sun exposure [4], but in this case, the right big toe skin tumor was only a compound nevus. Therefore, the primary site remains unknown. The prognosis of stage IV metastatic melanoma is very poor. We usually perform systemic management with dacarbazine and high-dose interleukin-2 (HD-IL-2) for treatment [5], but in this case, a laparoscopic omental tumor resection was necessary for the intraabdominal tumor due to the initial presentation of hemoperitoneum and hematoma. [6]

Establishing the diagnosis of metastatic melanoma can be difficult before operation. Cytological and pathological examinations, with the help of immunohistochemical staining, are central to diagnosis. Clinical examinations and imaging techniques are not specific. In most cases, treatment consists of surgical resection. The need for chemo-, radio-, or immunotherapy is case-dependent.

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# 原發部位不明之大網膜轉移性惡性黑色素瘤：案例報告

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**摘要**

大網膜轉移性惡性黑色素瘤非常罕見。過往，只有一個子宮惡性黑色素瘤併腹腔轉移的個案曾經被發表。我們提出一個原發部位不明之轉移性大網膜惡性黑色素瘤的病例報告，一名 58 歲男性患有腦動脈粥樣硬化，高血壓和第二型糖尿病。因急性右上腹悶痛持續數小時，來到急診求治。腹部 CT 顯示右下腹腸系膜有一個 6.5 公分的血腫。行腹腔鏡網膜腫瘤切除術。病理學報告為轉移性惡性黑色素瘤。免疫組織分析顯示黑色素瘤由大型黑素細胞組成，具有多形性和黑素色素沉積，HMB45 和 S100 蛋白染色呈陽性。原發部位可能來自於右側大腳趾的皮膚病變；為排除黑色素瘤，我們安排切片檢查，組織病理學診斷為複合痣。2 個月後出現惡性黑色素瘤細胞腹腔多處擴散，4 天後因多重器官衰竭死亡。

**關鍵詞：**轉移性大網膜惡性黑色素瘤、腹腔擴散

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Case Report

# Exaggerated Placental Site Lesion Arising from Antecedent Molar Pregnancy in a Perimenopausal Patient: A Case Report and Literature Review

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**Abstract**

Exaggerated placental site (EPS) lesions occur due to excessive infiltration of the implantation site by intermediate trophoblasts. These lesions may occur after a normal pregnancy, molar pregnancy, ectopic pregnancy, or abortion (whether induced or spontaneous). While EPS lesions share some features with placental site trophoblastic tumors (PSTTs), they are considered benign and trophoblastic in nature and typically do not require special treatment. In this case report, we present and discuss a rare case of an EPS lesion that arose from an antecedent complete hydatidiform mole in a 53-year-old perimenopausal woman. In addition, we review the related literature regarding EPS lesions.

**Key words:** exaggerated placental site lesion, molar pregnancy, placental site trophoblastic tumor

## Introduction

The cells present in exaggerated placental site (EPS) lesions, placental site nodules (PSNs), placental site trophoblastic tumors (PSTTs), and epithelioid trophoblastic tumors primarily consist of intermediate (extravillous) trophoblasts, with PSNs and EPS lesions representing two forms of benign pregnancy-related lesions. Both PSNs and EPS lesions can remain for some time after a normal pregnancy, miscarriage, or abortion and are characterized by abundant uninvoluted intermediate trophoblastic cells. However, while PSNs are discrete and well-defined lesions, EPS lesions are characterized by diffuse infiltration of the myometrium by intermediate trophoblasts at the implantation site, which gives them a diffuse infiltrative architecture similar to that of a normal placental site [1].

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The diffuse infiltration of the myometrium at the implantation site, as well as that of the associated vessels, is observed in both EPS lesions and PSTTs. However, PSTTs can be distinguished from EPS lesions in that PSTTs represent massively infiltrating lesions consisting of solid sheets and cords of cells that assume an epithelioid appearance [2]. In addition, while chorionic villi are typically found in EPS lesions, they are not generally associated with PSTTs.

In this paper, we present the case of a 53-year-old perimenopausal woman with an EPS lesion that arose from an antecedent molar pregnancy, with a focus on how the EPS lesion was differentially diagnosed from PSTT. In addition, it should be noted that intermediate trophoblastic cells can easily be confused with various neoplastic conditions due to their pleomorphism and physiological tendency to invade blood vessels and the myometrium. In particular, the potential for this confusion is high when these cells are found in unusual clinical situations, such as during perimenopause [3-6].

## Clinical case

A 53-year-old perimenopausal woman (gesta 3, para 3) was initially admitted to another hospital due to active uterine bleeding 4 weeks prior to being seen at our hospital. The patient had had a normal full-term pregnancy 24 years previously and had no known history of hormonal therapy during the previous 10 years. She underwent a dilatation and curettage (D&C) to investigate the etiology of her profuse bleeding. Microscopically, her tissue specimens showed the presence of chorionic villi, trophoblasts, decidual tissue, and blood clots. In addition, the villi exhibited marked hydropic changes with frequent cistern formation within the stroma. In addition, the outlines of the villi exhibited focally circumferential trophoblastic hyperplasia with intervillous trophoblast bridging. Moreover, moderate to marked trophoblastic atypia was also evident. The subsequent pathology report indicated the presence of a complete hydatidiform mole, also known as a molar pregnancy. Although the patient's beta-human chorionic gonadotropin (β-hCG) level had precipitously dropped from 55,326 IU/mL to 7,579 IU/mL beginning three weeks after the D&C, her uterine bleeding had continued.

Due to the prolonged bleeding, the patient was transferred to our hospital at 4 weeks after her D&C procedure for further treatment. At this time, her β-hCG level had risen to 9975 IU/mL. A pelvic ultrasound revealed a mass in her uterine cavity that was subsequently removed via a laparoscopically assisted vaginal hysterectomy (LAVH). The mass was later confirmed to be a single small polypoid tumor in the fundus measuring approximately 4.2 cm × 2.8 cm × 2.0 cm in size (Fig. 1). The patient's β-hCG level had dropped to a normal level two months after the LAVH.

Immunohistochemical (IHC) staining of the tumor tissue samples was indicative of an EPS lesion rather than a PSTT in that the cells were positive for both CK18 and human placental lactogen (hPL), negative for P63, and only focally positive for hCG (Fig. 2).

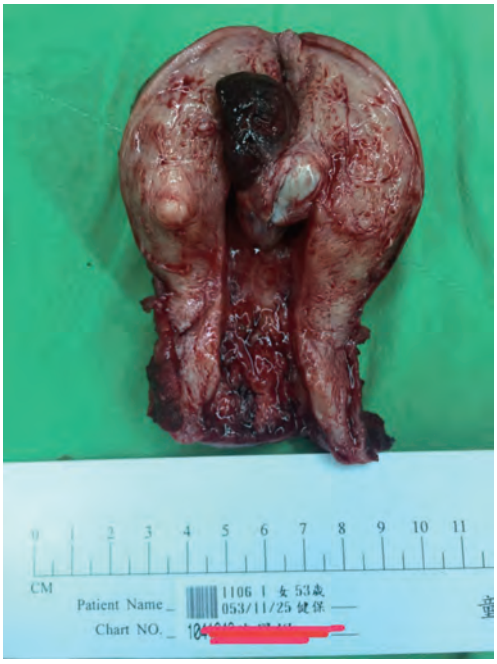


**Fig. 1** Specimen with a single small polypoid tumor in the fundus area, measuring approximately 4.2 × 2.8 × 2.0 cm.

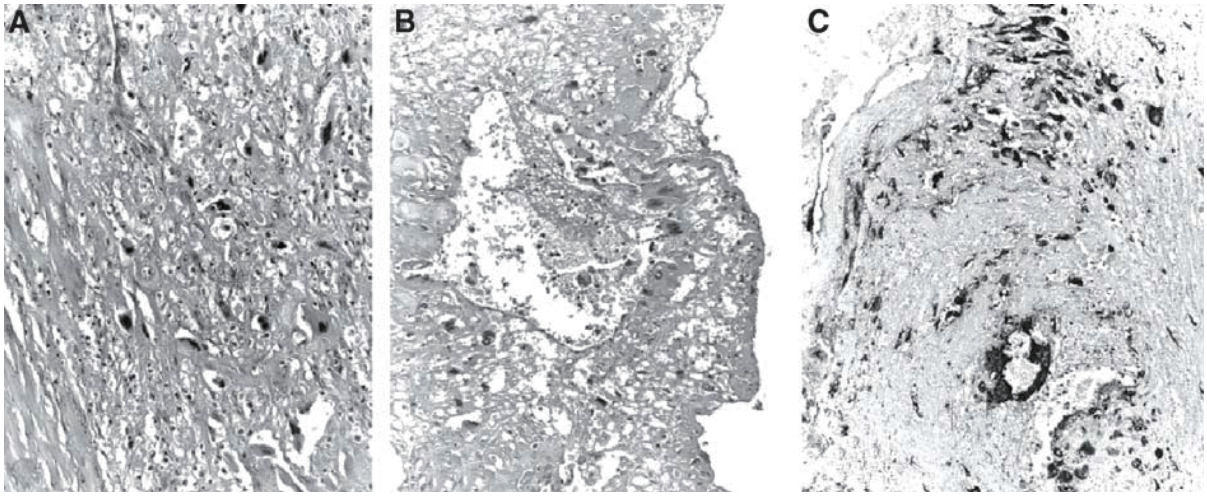


**Fig. 2** Microscopic tissue images showing numerous loose intermediate trophoblasts with nuclear pleomorphism (A), colonizing vascular walls (B), and marked positive for hPL (C).

In addition, the Ki-67 index was considered < 1%. This immunophenotype was regarded as characteristic of intermediate (extravillous) trophoblasts. Collectively, our findings supported the diagnosis of an EPS lesion that arose from an antecedent molar pregnancy.

## Discussion

In terms of morphological characteristics, EPS lesions generally display the outer extreme of the range of characteristics for a normal implantation site. In other words, while it is true that the placental site is typically exaggerated in cases of complete molar pregnancy, it is important to note that EPS lesions may also develop in a pregnancy involving normal gestation. Furthermore, an EPS lesion may occur in the aftermath of various circumstances, including a normal pregnancy, molar pregnancy, ectopic pregnancy with implantation in the fallopian tube, or an abortion, whether induced or spontaneous [7].

Among the primary characteristics of EPS lesions is a substantial increase in the size and number of individual intermediate trophoblasts. Because an EPS lesion can extensively infiltrate portions of the myometrium, curettage samples obtained in cases involving an EPS lesion will typically include at least a few tissue fragments containing parts of the lesion. A number of chorionic villi may also be expected in such tissue samples [8]. Moreover, the intermediate trophoblasts found in the EPS lesion itself would be expected to be more hyperchromatic and larger than normal intermediate trophoblasts. However, these trophoblastic cells do not exhibit mitotic activity. In cases in which the exaggerated implantation site results from an abortion, Ki-67 immunostaining will be undetectable.

While it is difficult to distinguish an EPS lesion from a PSTT, in both morphological and immunohistochemical terms, an EPS lesion is not a type of tumor. Rather, EPS lesions are simply an unusually conspicuous, but nonetheless, physiologic placental site [8]. However, because the cytological and immunophenotypical features of EPS cells and those of intermediate trophoblasts resulting from the neoplastic proliferation of PSTTs are quite similar, it is critical to make the differential diagnosis between EPS lesions and PSTT in the clinical setting.

An EPS lesion occurs as a result of the excessive infiltration of the implantation site by intermediate (extravillous) trophoblastic cells. These trophoblastic cells are distinct from chorionic-type and villous-type intermediate trophoblastic cells [9]. The main function of intermediate trophoblasts is to establish the maternofetal circulation during early pregnancy by invading the spiral arteries in the basal plate [10].

As mentioned above, EPS lesions share numerous features with PSTTs. However, there are various features that can be used to distinguish EPS lesions from PSTTs. In addition to the aforementioned fact that chorionic villi are typically found in EPS lesions but not associated with PSTTs, EPS lesions also differ from PSTTs in that they have no confluent growth or mitosis, and they are typically mixed with villous tissue and decidua [11]. In addition, a previous study found the Ki-67 index of intermediate trophoblasts near an EPS lesion to be close to zero, while that of intermediate trophoblasts at the molar implantation site of a molar pregnancy was 5.2% ± 4.0%, and that of intermediate trophoblasts in a PSTT was 14% ± 6.9% [12].

This difference in the Ki-67 index value between EPS lesions and PSTTs was important to the differential diagnosis in the present case due to other similarities between the two conditions. For example, a previous study found that only approximately 12% of PSTTs reported in the literature were preceded by molar pregnancy [13]. While a molar pregnancy had been diagnosed in this case, that did not rule out the possibility that the tumor was a PSTT. Moreover, while the average age of PSTT diagnosis is 30 years, patients with PSTT have ranged in age from as young as 19 years old to postmenopausal women more than 60 years old [13]. Therefore, the age and perimenopausal status of the patient in this case were not diagnostically conclusive.

Nonetheless, given our clinical findings and the known characteristics of EPS lesions versus PSTTs, the differential diagnosis of an EPS lesion arising from an antecedent molar pregnancy was determined in the present case. In particular, the presence of chorionic villi and decidual tissue in the tissue specimens, coupled with a Ki-67 index < 1%, indicated a diagnosis of EPS lesion as opposed to PSTT.

In the clinical setting, EPS lesions must be distinguished from the various intermediate trophoblastic tumors, as surgical intervention and/or chemotherapy would likely be required to treat the latter. In most cases of EPS lesions, the lesions are the result of

a physiological process and thus can remain for some time after a normal pregnancy. Typically, they do not require any specific treatment, although monitoring hCG levels is considered necessary. If active management is required, uterine artery embolization or hysteroscopic ablation can potentially be utilized. This is especially true in cases involving young patients that wish to have children in the future. In the present case, while the lesion was small and superficial, an LAVH was performed because the patient did not have a desire to preserve her fertility.

This case was of particular interest because it involved an EPS lesion that arose from an antecedent molar pregnancy in a 53-year-old woman who still had regular menses. EPS lesions are known to share some features with other types of lesions, including PSTTs. Nonetheless, EPS lesions can be distinguished according to various characteristics, including those revealed by immunohistochemistry, such as a very low Ki-67 index. Accordingly, it is important that clinicians be aware of and familiar with this pathology. In the event that a suspicious lesion is found at a placental site, a biopsy should be taken from the lesion for pathologic examination.

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# 停經前患者因妊娠葡萄胎引發之超常胎盤部位反應：病例報告與文獻回顧

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**摘要**

超常胎盤部位反應（exaggerated placental site，EPS）肇因於中型滋養層細胞於植入部位之過度浸潤，好發於正常妊娠、葡萄胎、異位妊娠、人工或自然流產。EPS 本身屬於良性和滋養性的病變，通常無需進行任何特殊治療，雖與胎盤滋養層細胞腫瘤（placental site trophoblastic tumor，PSTT）有些相似之處，仍具其明顯特性。本個案報告呈現的是一宗罕見 EPS 病例，一位 53 歲的停經前婦女因完全性妊娠葡萄胎病史引發之 EPS，同時並探討與 EPS 相關之文獻。

**關鍵詞：**超常胎盤部位反應、葡萄胎、胎盤滋養層細胞腫瘤

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Case Report

# Neonatal Abstinence Syndrome after Maternal Methadone Maintenance Treatment: A Case Report and Literature Review

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**Abstract**

Neonatal abstinence syndrome (NAS) is a serious condition affecting newborns that typically occurs as a result of opioid withdrawal following prenatal opioid exposure. It generally manifests as a range of symptoms impacting the autonomic nervous system, gastrointestinal tract, and central nervous system. If left untreated or treated inappropriately, NAS can result in severe complications, including death. Here, we present a case involving a newborn with NAS born to a mother undergoing methadone maintenance treatment (MMT) for prior heroin use. The diagnosis and treatment of NAS among babies, as well as dilemmas associated with the promotion of MMT by governmental authorities, are also discussed.

**Key words:** Neonatal abstinence syndrome, methadone maintenance treatment

## Introduction

Neonatal abstinence syndrome (NAS), initially named congenital morphinism, is a serious and highly variable condition that typically occurs due to abrupt discontinuation of chronic fetal exposure to substances, such as morphine, heroin, buprenorphine, antidepressants, anxiolytics, and/or others, which were used or abused by the mother during pregnancy [1]. NAS is characterized by signs and symptoms affecting the autonomic nervous system (diaphoresis, sweating, fever, and tachypnea), gastrointestinal tract (vomiting and diarrhea), and central nervous system (CNS) (tremors, myoclonus, seizure, elevated startle reflex, feeding difficulty, and abnormal sleep patterns) that occurs immediately after birth [2]. Moreover, if left untreated, the condition can result in severe complications and even death. Methadone, an artificial opiate derivative, has been used for the treatment of pregnant women addicted to heroin since the 1970s. The synthetic opiate blocks the euphoric effect of heroin in addicted individuals and eliminates their carving for drugs. Although methadone maintenance treatment (MMT) has been generally believed to promote better neonatal outcomes, reductions in heroin dosage and frequency of use during pregnancy, and improvements in overall maternal health [3], few reports have discussed the association between MMT and NAS.

Here, we present a case involving a newborn with NAS born to a mother who had formerly used heroin for several years and subsequently received MMT for her addiction, which was continued over the course of her pregnancy. Although most medical detox psychiatrists in Taiwan tend to recommend MMT for pregnant women addicted to opioids, this case suggests MMT during pregnancy can, and often does, lead to NAS, one of the untoward effects thereof.

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## Case Report

This case involved a male infant born at full term (gestational age: 37 5/7 weeks) to a healthy mother via spontaneous vaginal delivery. The baby, born to non-consanguineous parents, had a birth weight of 3420 g (50–75 percentile), body length of 51 cm (50–75 percentile), and head circumference of 35 cm (50–75 percentile). The baby's mother had been addicted to heroin for 10 years and had received MMT for her addiction for the preceding 5 years, with the treatment continuing during her pregnancy. She denied any heroin use and having experienced any fever or other symptoms of notable discomfort during pregnancy and tested negative for all hepatitis serum markers.

The male infant's initial Apgar scores were 8 and 9 at 1 and 5 min after birth, respectively. Physical examination revealed that he had an alert consciousness, a pinkish skin color, and a supple neck. A day after birth, intermittent perspiration, tremors, excessive Moro reflex, hypertonia, and myoclonic jerks were initially noted, which was temporarily alleviated by the intravenous administration of 0.2 mg/kg of diazepam. However, irritability, sweating, tachypnea [60–70 breaths/minute with oxygen desaturation (88%–92%) and mild subcostal retraction], tachycardia (120–146 beats/min), exaggerated tremors, Moro reflex, hypertonia, and myoclonic jerks were frequently observed. Electroencephalography (EEG) results did not show any epileptiform discharge. Considering the aforementioned symptoms, a tentative diagnosis of NAS was established, and the baby was transferred to the sick baby room for further evaluation and management.

The severity of the infant's withdrawal symptoms was assessed using the modified Finnegan scoring system (FSS), which lists 21 symptoms frequently observed among opiate-exposed infants (Table 1). Each symptom and its associated degree of severity are assigned a score, and the total abstinence score is determined by summing the scores assigned for each symptom over the scoring period. As shown in Fig. 1A, the baby's score increased from 0 to 3 on the day of birth and from 3 to 6 a day thereafter. Diazepam administration did not improve his irritability. Despite subsequently decreasing light and noise exposure, increasing swaddling and holding, encouraging breastfeeding and frequent non-nutritive sucking, and administering a phenobarbital dose of 16 mg/kg, his scores further increased to 8 on the third day and reached a maximum of 11 on the fifth day before gradually decreasing thereafter (Fig. 1A). After stabilization of his vital signs (Fig. 1B–D) and abatement of tachypnea and other NAS symptoms, he was discharged from the hospital on the eleventh day. Follow-up after 9 months revealed no signs and symptoms related to NAS. The child is currently

**Table 1.** modified Finnegan scoring system (FSS)

| Signs & Symptoms | Scores |
|---|---|
| Central Nervous System Disturbances | |
| Excessive crying | 2 |
| Continue high pitched crying | 3 |
| Sleeps < 3 Hr after feeding | 1 |
| Sleeps < 2 Hr after feeding | 2 |
| Sleeps < 1 Hr after feeding | 3 |
| Hyperactive Moro reflex | 2 |
| Markedly hyperactive Moro reflex | 3 |
| Mild tremors: disturbed | 1 |
| Moderate to severe tremors: disturbed | 2 |
| Mild tremors: undisturbed | 3 |
| Moderate to severe tremors undisturbed | 4 |
| Increased muscle tone | 2 |
| Excoriation | 1 |
| Myoclonic jerk | 3 |
| Generalized convulsions | |
| Gastrointestinal Disturbances | |
| Excessive sucking | 1 |
| Poor feeding | 2 |
| Regurgitation | 2 |
| Projectile vomiting | 3 |
| Loose stools | 2 |
| Watery stools | 3 |
| Others | |
| Sweating | 1 |
| Fever 37.3-38.3℃ | 1 |
| Fever ≧ 38.4℃ | 2 |
| Frequent yawning (>3-4/30 mins) | 1 |
| Mottling | 1 |
| Nasal stuffiness | 1 |
| Sneezing (>3-4/30 mins) | 2 |
| Nasal flaring | 1 |
| Respiratory rate (> 60/min) | 1 |
| Respiratory rate (>60/min with retractions) | 2 |

undergoing a rehabilitation program for delayed fine motor development.

## Discussion

The clinical characteristics of NAS that usually appear first include irritability, jitteriness, tremors, and excessive crying. Moreover, heart rate, respiratory rate, muscle tone, and other physiologic responses to stimuli become impaired due to the autonomic nervous system dysfunction. Accordingly, tachypnea, nasal flaring, and nasal stuffiness among newborns may be misinterpreted as respiratory distress, while poor feeding, excessive motor activity, regurgitation, vomiting, diarrhea, and hyperthermia can lead infants to be misdiagnosed with sepsis. Occasionally, seizures and serious withdrawal manifestations may occur insidiously for which emergency treatment may be needed. In the present case, most of the aforementioned signs and symptoms of NAS, except for seizures, were noted. Considering that the doctor on duty might not be familiar with NAS, diazepam might have been administered to temporarily alleviated the baby's discomfort. However, the persistent vital sign abnormalities served a clues to the correct diagnosis of NAS. Table 1 had been modified from the FSS, which is commonly used for opioid withdrawal assessment and FSS helps clinicians monitor, titrate, and terminate therapy. Quantifying the severity of NAS assists in determining if and when pharmacological intervention will be needed [4].

Opioids mostly act through opioid receptors (G protein-coupled receptors, μ, κ, and δ), which are extensively distributed across the central and peripheral nervous system, gastrointestinal system, and various other systems. Methadone use during pregnancy was initially believed to be unassociated with withdrawal among neonates; however, subsequent experiences contradicted this initial misimpression. The water solubility and lipophilicity of methadone allows it to easily cross the placenta and reach the fetus. Moreover, the transmission of opioids across the placenta has been found to increase as gestation progresses. The lack of opioids in a chronically

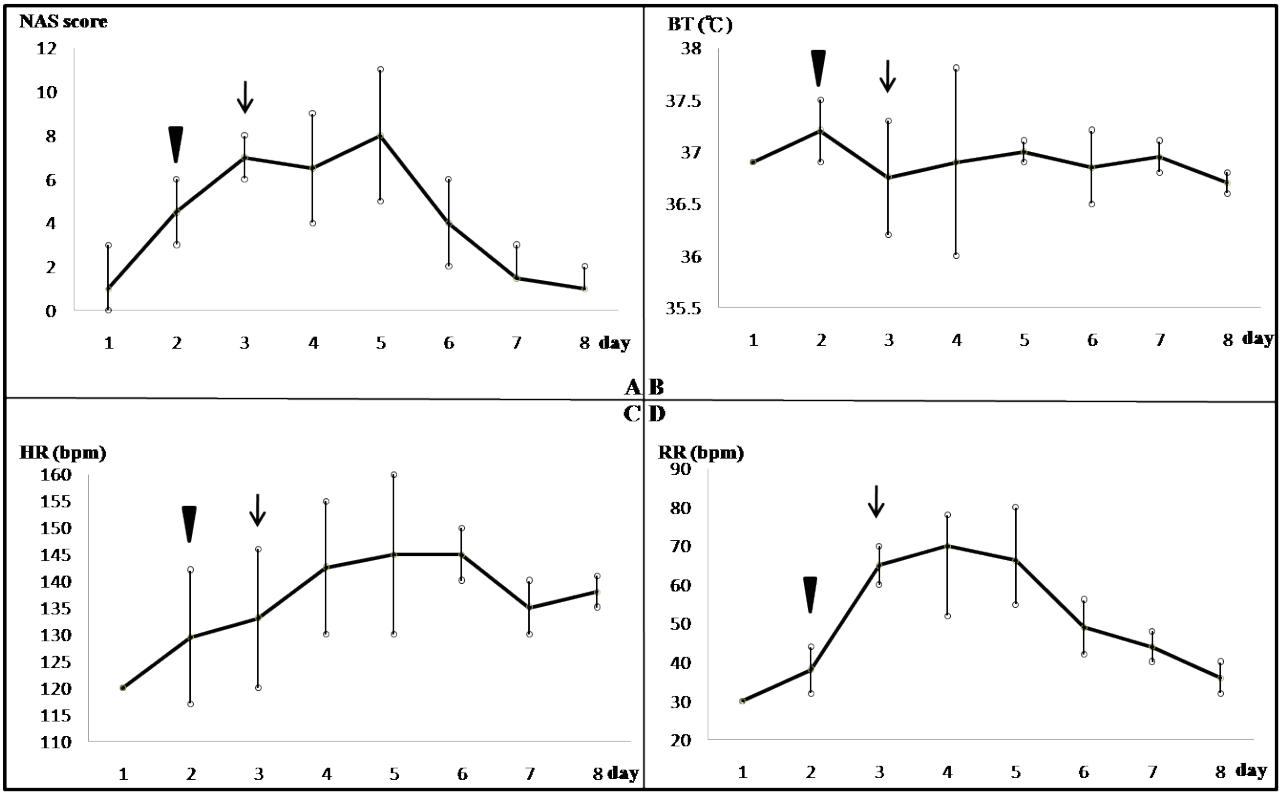


**Fig. 1** Graphic visualization of the daily neonatal abstinence syndrome scores (Fig. 1A) and vital signs, including body temperatures (BT; Fig. 1B), heart rates (HR; Fig 1C), and respiratory rates (RR; Fig. 1D), of the present case from the date of birth. ▼: 0.2 mg/kg of diazepam injection. ↙: 16 mg/kg of phenobarbital injection.

stimulated state increases opioid receptor activity, triggering various excessive neurotransmitters through a cascade of enzymatic activities and enormous cellular ionic imbalance, leading to NAS. However, NAS is much more complex because of immature neurologic development, impaired neurologic processing, and complex materno-feto-placental pharmacokinetics [5]. Clinically, tremors, exaggerated Moro reflex, hypertonia, and myoclonic jerks are more common during methadone withdrawal. An EEG may be required to differentiate them from seizures.

The treatment of NAS includes limiting light and noise exposure, promoting swaddling and holding, and providing opportunities for non-nutritive sucking, breastfeeding, and adequate nutrition to minimize weight loss, as well as rooming-in. Phenobarbital has been used as a single therapeutic agent or as an adjunct to morphine or methadone for NAS. Although the dosage of phenobarbital for NAS does not prevent seizures and improve gastrointestinal symptoms, its advantages include the following: (1) can be used as an adjuvant, (2) allows for serum level monitoring, and (3) decreases the duration of morphine or methadone treatment. While morphine decreases the incidence of seizures, improves feeding and diarrhea, and decreases agitation, its disadvantages include increased risk for sedation and respiratory depression and prolonged hospitalization. Buprenorphine is a new option for the treatment of NAS; however, no large-scale studies have been available to support its use. Sedatives, such as diazepam and chlorpromazine, are impractical due to their prolonged half-lives and associated complications. Opioid antagonists, such as naloxone, are contraindicated given that they may precipitate seizures in neonates. Despite numerous options, no standardized regimens for the management of NAS have currently been established owing to the absence of large-scale studies comparing such medications and variations in the spectrum of withdrawal for different drugs, doses, weights, and gestational periods [6].

While most health authorities claim that MMT can minimize the likelihood of NAS occurrence [7], ironically, MMT among pregnant women addicted to opioids does carry risks. Specifically, several studies have found that MMT carries increased risk for NAS among the babies born to such women. For example, a multisite cohort study conducted by Ordean et al. showed that pharmacological care was required for 27% of the neonates born to women receiving MMT due to a history of opioid use disorder [8], while other studies have reported even higher rates of 50% and 60% [9,10]. Moreover, incidence rates of NAS is only likely to increase given the general prevalence of opioid use and the recent increase in perinatal opioid use across many countries. Such an increase in opioid use has likewise been noted in Taiwan, a trend which underscores the need for both physicians and patients to be aware of the risks associated with MMT during pregnancy.

Despite the apparent risk for NAS brought by MMT, Taiwanese authorities have assured pregnant women addicted to opioids that MMT poses no risks to their babies while tending to ignore the threat. Although such assurances are presumably part of a well-intentioned effort to encourage such women to avoid the even greater dangers posed by heroin use during pregnancy, they may have the unintended consequence of damaging the trust women addicted to opioids have in their health care providers, which could in turn cause them to reject necessary treatments and guidance from such providers. We believe that the best course of action for addiction specialists would be to inform pregnant patients who are willing to undergo detoxification and MMT about the risk of NAS. Greater openness regarding the subject of NAS could help pregnant women receiving MMT better prepare for any NAS-related treatment required after giving birth.

The number of individuals with a drug addiction in Taiwan has continued to increase, with amphetamine, ketamine, and 3,4-methylenedioxymethamphetamine (MDMA) having become the most commonly abused illicit drugs. Accordingly, the incidence of NAS has also been rising. However, most pediatricians are not familiar with NAS and are not adept in its diagnosis, especially among parents of infants with NAS who had received/are receiving MMT, which the government claims to be "safe". Considering that NAS can potentially be fatal, we believe that the circulation of this case report will be of considerable benefit to all concerned.

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# 母親孕期使用美沙酮戒毒引發新生兒戒斷症候群一病歷報告與文獻回顧

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**摘要**

新生兒戒斷症候群是一種因新生兒在胎兒時期暴露於鴉片類藥物的環境，隨出生後鴉片類藥物的終止，隨即出現會嚴重影響新生兒的情況。此症候群會表現出影響自主神經系統、腸胃道及中樞神經系統的症狀。如果不治療或處理不當，新生兒戒斷症候群可能會有嚴重的併發症，甚至是死亡。我們報告一個患有新生兒戒斷症候群的嬰兒，他的母親之前有使用海洛因，在懷孕前與懷孕期間接受美沙酮維持治療。本文將介紹由新生兒戒斷症候群的診斷與治療，以及討論美沙酮維持治療有關的困境。

**關鍵詞：**新生兒戒斷症候群、美沙酮維持治療

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Image

# Esophageal Cancer Presenting as Upper Airway Obstruction in a 75-year-old Man with Chronic Obstructive Pulmonary Disease

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**Abstract**

Esophageal cancer typically presents as dysphagia and weight loss but can also present as upper airway obstruction, which is a medical emergency. Here we present a case of a 75-year-old man with a history of chronic obstructive pulmonary disease refractory to medical treatment. Central airway obstruction was suspected. Computerized tomography of the chest showed an upper tracheal tumor. Bronchoscopy showed no endoluminal lesion but with airway obstruction from external compression. Upper gastrointestinal endoscopy showed a submucosal mass over the upper esophagus with lumen stenosis. Pathology showed squamous cell carcinoma of the esophagus. Endotracheal intubation was performed, and tracheostomy was done to bypass stenotic lesion. Chemotherapy was attempted due to the high risk of surgery.

**Key words:** esophageal cancer, upper airway obstruction, chronic obstructive pulmonary disease

## Introduction

A 75-year-old man presented to the emergency department with progressive shortness of breath and a productive cough for two weeks. He also had throat discomfort, dysphagia, and body weight loss. Upon physical examination, he was clearly conscious and oriented, yet in respiratory distress. His body temperature was 36.7°C. He had a heart rate of 94 beats per minute, a respiratory rate of 29 breaths per minute, and a blood pressure of 150/77 mmHg and respiratory accessory muscls usage, auscultation of lungs showed diffuse bilateral expiratory wheezes. These indicated chronic obstructive pulmonary disease with acute exacerbation. A pulmonary function test showed a post-bronchodilator FEV1 of 0.72 L (39% of predicted value, Gold stage 3). Despite receiving an intravenous steroid, inhalational bronchodilators, and an empiric antibiotic, the patient's dyspnea persisted. Computerized tomography of the chest with contrast showed a 4.2-centimeter hypoenhanced irregular mass in the posterior aspect of the upper trachea, adhesion to the upper esophagus, and a 1.8-centimeter irregular nodule in the right aspect of the upper esophagus, consider regional lymphadenopathies (Panel A). Retrospectively, a lateral view chest x-ray showed bulging mass over the trachea, revealing an esophageal mass. (Panel B). The patient was transferred to the medical intensive care unit due to impending respiratory failure. Bronchoscopic guided nasoendotracheal intubation was performed; no endoluminal lesion was found, but the patient did have airway obstruction from external compression (Panel C). Upper gastrointestinal endoscopy showed a bulging submucosal mass (arrowhead) over the upper esophagus with partial lumen stenosis, and a biopsy

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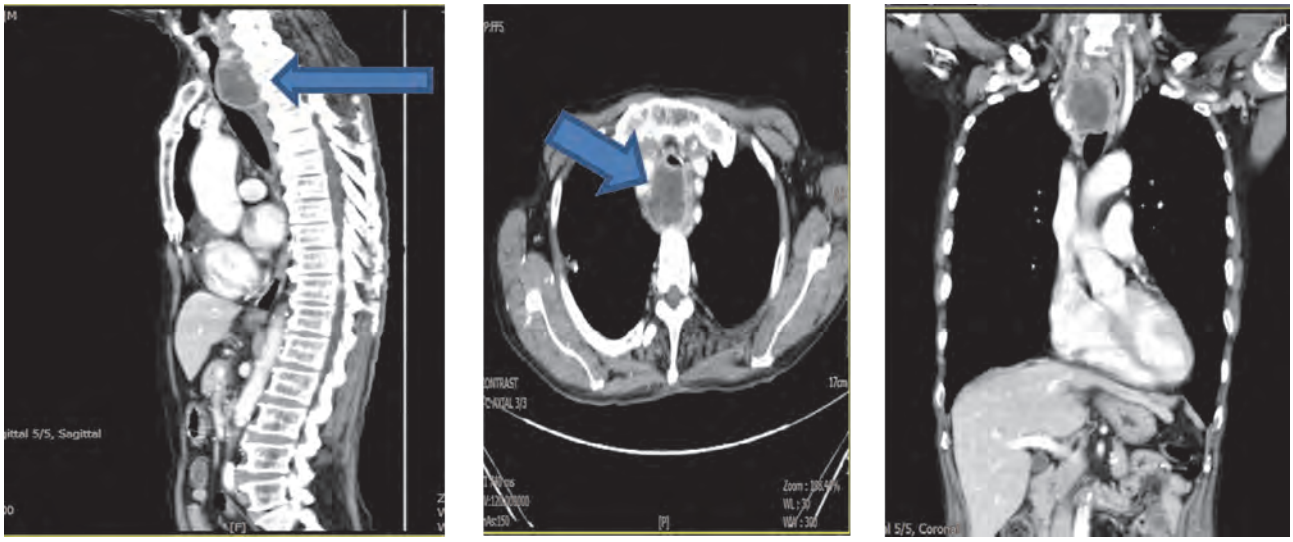
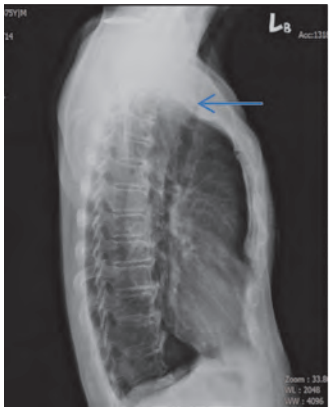
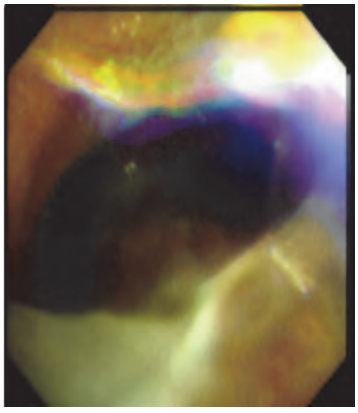


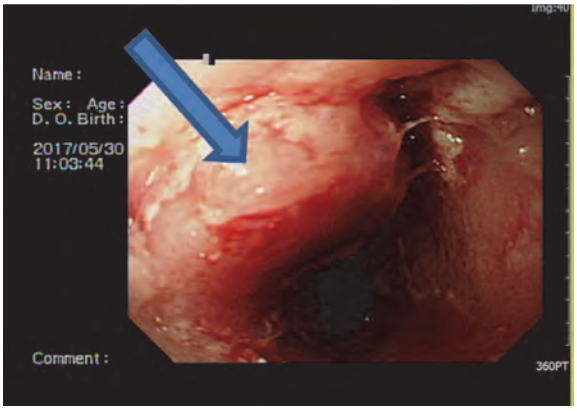
Panel A



Panel B



Panel C




Panel D

of the lesion was performed (Panel D). Pathology showed squamous cell carcinoma, which was keratinized and moderately differentiated. A tracheostomy was performed to bypass the tracheal tumor, but the patient and his relatives refused removal of tumor. Chemotherapy was attempted, but the patient responded poorly and passed away after approximately 3 months.

## Discussion

Initially, the patient's dyspnea was attributed to his underlying chronic obstructive pulmonary disease. After reevaluation, stridor during inspiration and expiration was noted especially in the supine position and upper airway obstruction was highly suspected. Central airway obstruction (CAO) refers to the obstruction of air flow in the trachea and mainstem bronchi. The most common causes of malignant CAO are direct extension into the airway lumen by extrinsic tumors. Of these tumors, the most common types are bronchogenic carcinomas (i.e., small cell lung cancer and non-small cell lung cancer), followed by esophageal and thyroid carcinomas. The most commonly encountered causes of nonmalignant CAO include granulation tissue resulting from prior endotracheal/tracheostomy tubes, airway foreign bodies, and tracheomalacia or bronchomalacia. Distant tumors may also metastasize to the airway, with the most common causes including renal cell, breast, and thyroid carcinoma[1].

Clinical manifestations of malignant CAO depend on the size, location, and rate of progression of airway obstruction[2]. Although wheezing indicates airflow through a narrowed orifice, its location does not always conform to the site of airflow obstruction.

Wheezing heard best over the trachea does not necessarily indicate that the trachea is the source of the obstruction. Unilateral wheezing, however, often suggests airway obstruction distal to the carina. The presence of persistent unilateral wheezing should always prompt the investigation of focal airway obstruction. Stridor is a sign of severe laryngeal or tracheal obstruction. Patients may also present with other nonspecific symptoms such as exertional dyspnea and positional wheezing. With an anatomically fixed obstruction, shortness of breath and wheezing are typically unresponsive to bronchodilators, and failure of a patient to improve with these measures should prompt the physician to consider the presence of CAO[3]. The airway often requires initial stabilization as a bridge to definitive management[4].

Treatment options for esophageal cancer include surgery, chemotherapy, and radiation therapy. Surgical resection may be considered in patients likely to tolerate surgery who present with benign diseases or resectable malignancies. Radiation therapy alone has been used for inoperable squamous cell carcinoma. For patients who are unable to tolerate initial chemoradiotherapy or who have a short estimated life expectancy (i.e., six months or less), we suggest alternative approaches to palliate dysphagia, such as endoscopic therapy (stenting, laser resection, photodynamic therapy [PDT], etc.) or brachytherapy, rather than concurrent chemoradiotherapy.

Due to the close anatomical relationship between the upper esophagus and the tracheobronchial tree, many patients with advanced esophageal cancer suffer from airway complications. These include airway stenosis or esophago-respiratory fistulation. Airway stenting is proven to offer effective palliation for patients with these complications[5].

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# 食道癌表現為上呼吸道阻塞在一位七十五歲有慢性阻塞性肺疾病男性病患

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**摘要**

食道癌常見的症狀包括吞嚥困難與體重減輕 , 少數表現為上呼吸道阻塞 , 而且是急症。我們提出一例 75 歲男性病患因呼吸困難，痰多，被診斷慢性阻塞性肺病急性發作，對藥物治療無效。進一步電腦斷層掃描撿查發現疑似氣管腫瘤，支氣管鏡卻沒發現氣管腫瘤但有支氣管壓迫性阻塞。進一步胃鏡撿查發現粘膜下腫瘤病理報告為鱗狀上皮癌。病患有接受氣管內插管。後續做氣管造口術及化療。

**關鍵詞：**食道癌、上呼吸道阻塞、慢性阻塞性肺病、氣管造口術

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Image

# Image on Clinical Medicine: Capillary Telangiectasia in Juvenile Dermatomyositis

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**Abstract**

Juvenile dermatomyositis (JDM) is the early-onset form of dermatomyositis, a systemic, autoimmune inflammatory muscle disorder characterized by proximal muscle weakness, characteristic skin lesions, and systemic manifestations. The course of JDM is highly variable: Some patients go into remission within 2–3 years, whereas others followed a cyclic course marked by relapses. The aim of treatment is to reduce long-term morbidity and restore physical function. High-dose corticosteroids are the mainstay of treatment, and dose tapering after a few weeks of therapy depends on the patient response. In this imaging report, we present the case of a 10-year-old girl diagnosed with JDM based on the classical findings of heliotrope rash, Gottron's papules, poikilodermatomyositis, elevated skeletal muscle enzyme levels, bilateral proximal muscle weakness, and denervation myopathy of the left rectus femoris and biceps muscles. Additionally, periungual erythema and telangiectases, filamentous or spider-like capillary changes were observed on bilateral hands by nailfold capillaroscopy.

**Key words:** juvenile dermatomyositis, periungual telangiectasias, poikiloderma

A 10-year-old girl presented with marked femoral muscle weakness resulting in difficulty in standing from a sitting position for two weeks. On presentation, heliotrope rash with asymmetric distribution was observed on her face. Additionally, two poikilodermatomyositis lesions defined as circumscribed areas of violaceous erythema were present over the left preauricular area. Periungual erythema and telangiectases were visible in both hands (Fig. A, arrows). By capillaroscopy, small, dilated, torsive vessels with a cobweb-like pattern were observed around the nailfolds (Fig. B). Gottron's papules were also found on bilateral knees and elbows.

An elevated creatine phosphokinase of 4811U/L was notable at presentation. A positive antinuclear antibody titer of 1:320 with a homogenous nuclear staining pattern was also observed; however, the levels of other immunopathological markers including extractable nuclear antigens and the myositis-specific antibodies (anti-Jo-1, anti-SRP, and anti-Mi-2) were within the normal range. Electromyogram revealed denervation myopathy of the left rectus femoris and biceps muscles. Therefore, the patient was definitively diagnosed with juvenile dermatomyositis (JDM) and received three courses of intravenous pulse methylprednisolone. Three months later, the creatine phosphokinase level was reduced to 407 U/L, and her muscle strength had improved.

JDM, which has an estimated annual incidence of 2–3 cases per million[1], is the most common idiopathic inflammatory myopathy of childhood and is characterized by proximal muscle weakness, evocative skin lesions, and systemic manifestations.

At present, the most widely used diagnostic criteria for JDM, defined by Bohan and Peter in 1975, are based on the presence of characteristic skin rash and

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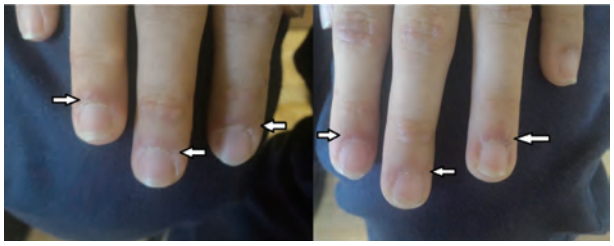

**Fig. A** Periungual telangiectasia. Dilated and tortuous blood vessels and mild atrophy with cuticular overgrowth observed around the base of the nail plate.

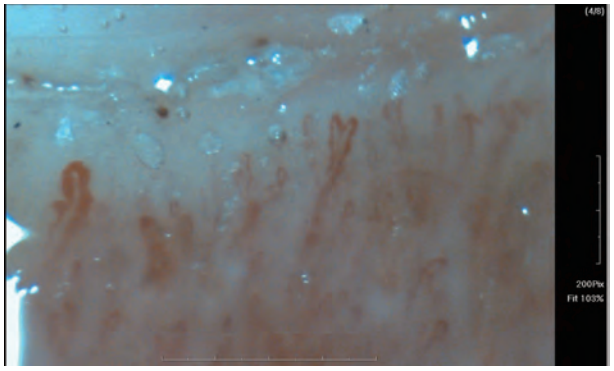

**Fig. B** Periungual telangiectasia. Dilated and discrete hairpin-like capillary loops at the base of the fingernails.

three of the following features: symmetric proximal muscle weakness, raised serum muscle enzymes, abnormal findings on muscle biopsy, and myopathic changes by electromyogram[2]. The current patient exhibited the pathognomonic manifestations of JDM including Gottron's papules overlying the dorsal interphalangeal or metacarpophalangeal areas, elbow, or knee joints[3]. Additionally, poikiloderma atrophicans vasculare, i.e., poikilodermatomyositis, a circumscribed violaceous erythema with associated telangiectasia, hypopigmentation, and superficial atrophy, is most commonly found over the posterior shoulders, back, and buttocks and as a V-shaped distribution over the anterior neck and chest[3]. Heliotrope rash, referred to as a violaceous coloration along eyelids, with periorbital edema, is considered a typical finding of dermatomyositis. Moreover, microscopical examination of the nailfold reveals markedly dilated capillaries with bushy loops in the attached proximal nail plate. The red-purple linear lesions, around 1 millimeter in width and several millimeters in length, are denoted as periungual telangiectasias, which reflect the vasculopathy associated with dermatomyositis, particularly in its juvenile form.

The primary treatment in JDM aims to reduce long-term morbidity and restore physical function. According to the most current evidence-based management[4], the effective and safe JDM treatment is high-dose corticosteroids, even intravenous methylprednisolone, in combination with either cyclosporin or methotrexate. Furthermore, the reported median treatment length to achieve disease remission is 41.9 months[4].

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# 臨床影像判讀：毛細微血管擴張症於青少年型皮肌炎

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**摘要**

青少年型皮肌炎，是一種系統系的自體免疫肌肉發炎疾病，以近端肢體無力以及特殊的皮膚病灶為主要表現，是皮肌炎的一種早發形式。其病程進展可長可短，可以在二至三年內病情緩減，亦可能週期性反覆發作。此疾病的治療目標首重減少長期發病率以及重建生理功能。治療初期仍以高劑量類固醇作為治療主流，之後視病人反應而做濃度調整。在這個影像報告中，該名確診為青少年型皮肌炎十歲女性，除依據典型皮膚表現，包括淡紫色皮疹、Gottron 氏丘疹、肌肉酵素的增加，以及左股直肌和左股二頭肌的去神經肌病變。而其雙側手指甲床及甲周變化，更可發現微血管呈現絲狀或蛛網狀的毛細血管改變，壓後可見動脈性搏動。

**關鍵詞：**青少年型皮肌炎、甲周血管擴張症、多形性皮炎

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## 貳、寫作原則

1. 原著論文（Original Articles）按下列順序撰寫：摘要、前言、材料與方法、結果、討論與結論、誌謝、參考文獻、附表、圖片說明、圖片（含照片）。每篇字數 3000 字以內，摘要 300 字以內，參考文獻 40 篇以內。
2. 病例報告（Case Reports）按下列順序撰寫：摘要、前言、病例、討論、參考文獻、附表、圖片說明、附圖、照片。凡病患顏面部位之相片必須遮去眼睛部位，表示尊重隱私。診療資料或臨床經過之圖表，原則上均限六個月以內。每篇字數 1500 字以內，摘要 150 字以內，參考文獻 10 篇以內。
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)、<br>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.)

## 伍、著作權

若著作人投稿於本刊經收錄後，同意授權本刊得再授權國家圖書館或其他資料庫業者，進行重製、透過網路提供服務、授權用户下載、列印、瀏覽等行爲。並得爲符合各資料庫之需求，酌作格式之修改。若爲摘譯、譯稿或改寫稿，需附原作者之正本同意書，並附原文影本一份；來稿如涉及版權，概由作者自負文責。

# 童綜合醫學雜誌

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## 綜　論



## 原　著



## 病例報告

## 影像判讀

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