Original paper

Pulmonary coin lesion caused by Dirofilaria immitis – a report of two cases with a minireview of the literature

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Dirofilariasis is a rare zoonosis, transmitted from infested dogs or other carnivorous animals to humans via mosquitoes.
Two male patients with a solitary, peripheral, well-defined, coin-like pulmonary lesion in the right upper lobe were presented.
Rapid enlargement of the lesion within a few months suggested malignancy, resulting in surgical removal. Microscopic examination of the resected lung revealed necrotic circumscribed lesions with embolized parasites in the vessels.
Both parasites were females of the species *Dirofilaria immitis*. They represent the first reported cases of pulmonary dirofilariasis in Slovenia. Awareness of this entity is important in the differential diagnosis of pulmonary coin lesions.

Key words: zoonosis, pulmonary dirofilariasis, coin lesion.

Introduction

Helminthic infections in humans are prevalent worldwide, but roundworms are only rarely detected in the lung. Human pulmonary dirofilariasis (HPD) is a rare zoonotic disease, most commonly caused by the filarial nematode *Dirofilaria immitis*. The infection is usually asymptomatic and presents on routine chest X-ray as a solitary pulmonary nodule suggestive of malignancy [1].

We present 2 patients with pulmonary dirofilariasis. They are the first documented cases of this uncommon disease in Slovenia.

Material and methods

An asymptomatic, peripheral, well-circumscribed mass was discovered by chest X-ray in the right upper lobe of the 42-year-old and 71-year-old men. Both patients underwent a preoperative clinical examination. Fine-needle aspiration biopsy, and bronchoscopy with bronchial and transbronchial biopsy were unsuccessful. Both nodules were clinically suspicious for malignancy resulting in excision. Frozen sections were performed in both cases. The remaining tissue obtained at surgery was stained with H&E, Weigert Van Gieson, periodic acid-Schiff (PAS), Ziehl-Neelsen, and Grocott silver stainings.

Results

Patient 1

A 71-year-old retired driver with a 30-year history of smoking one pack of cigarettes per day presented with repeated respiratory infections, high temperature (38.5–39°C), shivers, malaise, and productive cough for 4 months before admission. The patient denied haemoptysis, chest pain, or hoarseness. His peripheral blood revealed a slightly elevated erythrocyte sedimentation rate and leukocytosis without anaemia or eosinophilia. A subsequent chest X-ray showed solitary, peripheral, well-defined pulmonary opacity in the right upper lobe, an oval pulmonary nodular lesion in the posterior seg-

ment of the right upper pulmonary lobe, measuring around 15 mm (Fig. 1). The lesion was below the pleural surface with ribbon-like extensions into the surrounding lung parenchyma. Computer tomography was not performed. Fine-needle aspiration biopsy disclosed squamous metaplasia with necrotic debris in the background. Transbronchial biopsy revealed chronic catarrhal bronchitis with focal metaplastic changes of bronchial mucosa with moderate dysplasia and inflammation. A wedge-shaped resection of the upper right lobe was performed. Frozen sections showed an acellular necrotic area and peripheral inflammation suggesting tuberculoma. In the resected lobe, a well-circumscribed nodule was found, measuring 15 mm in diameter. On cut section, the nodule appeared granular grey-yellow and slightly softened in the central portion.

Microscopically, there was subtotal necrosis surrounded by fibrous pseudocapsule including few multinuclear giant cells, suggesting the diagnosis of tuberculosis (Fig. 2A). Differential diagnosis also included echinococcosis due to preserved partially fragmented laminated structures. Evaluation of infarcted tissue and the embolized lung vessel, however, disclosed cross-sectioned helminth. Parasite, better seen with Weigert Van Gieson staining, measured 170–260 μm in diameter (Fig. 2B). The observed circular muscular layer, central intestinal tube, pair of reproductive organs (uterus), and smooth laminated chitin-like cuticle without longitudinal ridges confirmed the diagnosis of female *Dirofilaria immitis*.

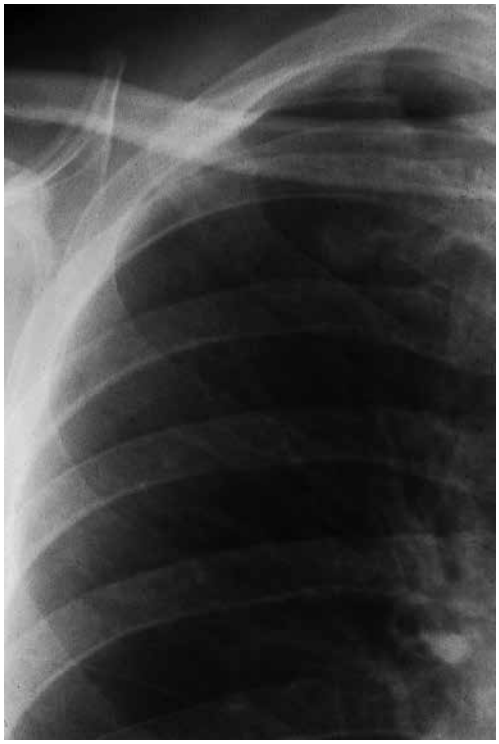

Fig. 1. Chest radiograph revealing solitary peripheral opacity ('coin lesion') in the right upper lobe

Patient 2

A 41-year-old male was admitted for routine medical examination and a chest X-ray. The patient denied chest pain, dyspnoea, wheezing, fevers, night sweats, or weight loss. He also denied alcohol abuse or smoking. Lungs were clear to auscultation. The chest radiograph revealed a solitary non-calcified subpleural nodule in the apical region of the right upper lung lobe, measuring 15 mm in diameter. Fine-needle aspiration biopsy and TTB were both non-specific, showing only a few inflammatory cells and fibrous tissue. The lesion was considered as a benign solitary fibrous tumour of the pleura, and surgical resection was not performed. The patient was asymptomatic and followed-up and re-examined every 3 months with chest X-ray. Nine months later, the nodule suddenly enlarged to 25 mm in diameter, prompting surgical removal. Fro-

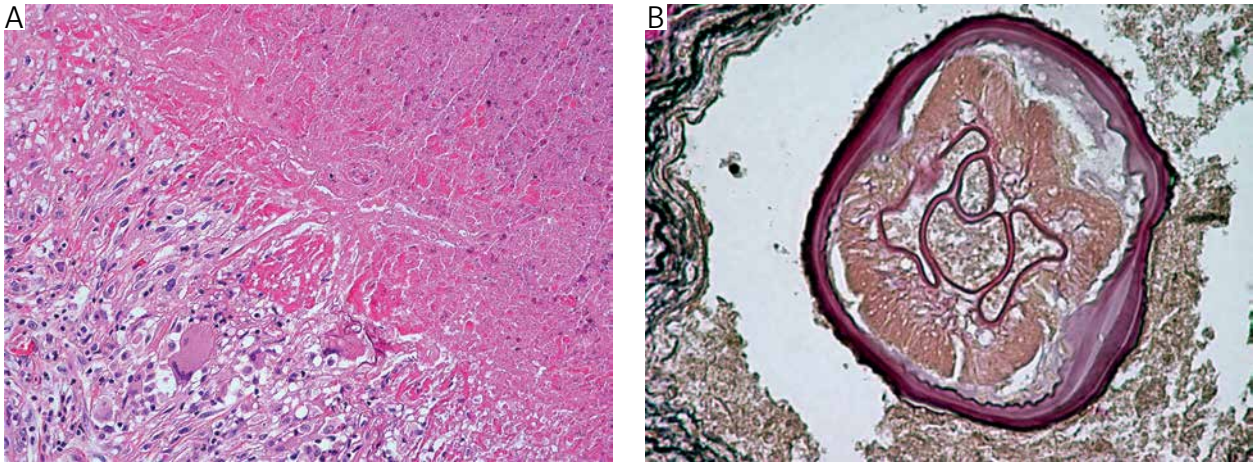

Fig. 2. A) Necrosis surrounded by fibrous pseudocapsule with multinuclear giant cells and inflammation. HE, magnification 100×; B) Transverse section through an immature adult of *Dirofilaria* species, with Weigert Van Gieson staining. The circular muscular layer, central intestinal tube, pair of reproductive organs (uterus), and smooth cuticle. Weigert Van Gieson, magnification 400×

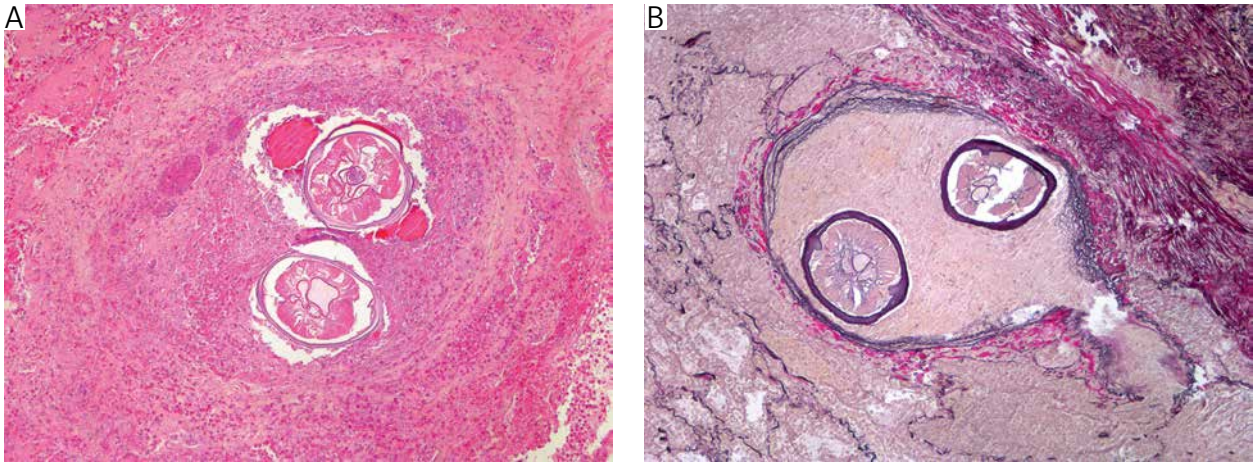

Fig. 3. A) Infarcted tissue and cross-sectioned helminth. HE, magnification 200×; B) Haemorrhagic infarction, embolized lung vessel with helminth, better visualized with Weigert Van Gieson, magnification 200×

zen section was performed during surgery, suggesting pulmonary tuberculosis due to abundant central necrosis surrounded by fibrous pseudocapsule and few Langhans' giant cells. In the centre of the resected tissue, there was a well circumscribed, soft, greyish nodule with a reddish rim. Microscopical examination demonstrated haemorrhagic infarction with dirofilarial embolization of pulmonary vessel (Figs. 3A, B), similar to Patient 1.

Discussion

Pulmonary dirofilariasis is caused worldwide by *Dirofilaria immitis* and occasionally by *Dirofilaria repens*. Humans are accidental and dead-end hosts of *Dirofilaria* organisms, which are transmitted from dogs and other carnivores to humans by mosquitoes infected with the third-stage larvae [1, 2].

The age span of patients is wide, at 8–80 years, with an average age of 51–58 years. Males are involved in 59–70%, females in 30–40%. More than half of all patients are asymptomatic; the remaining patients present with cough, fever, chest pain, haemoptysis, and arthralgias. In almost 90% of reported cases, lesions are solitary, noncalcified, well circumscribed, and peripheral. Approximately three-quarters of nodules are in the right lung, with a predilection for the lower lobe. Sub-pleural lesions are encountered in 68% of all cases and may be associated with pleural effusion [1, 3, 4].

Because most of the injected larvae perish at the site of inoculation, the parasite usually cannot be identified in the blood. Dashiell described the first report of HPD due to *Dirofilaria immitis* in 1961 [5].

Dirofilaria includes more than 40 species, but only a few infect humans. In 2016 around 400 cases worldwide and only about 30 cases of Dirofilaria immitis infection of humans in Europe had been reported, which were demonstrated as lung or intravascular or heart tumorous lesions [6–12]. Until now only a few more cases of HPD in Europe have been reported [13, 14]. This confirms that in Europe, pulmonary dirofilariasis is very rare medical entity.

Infections in humans have been reported in both temperate and tropical regions of Asia, Australia, Japan, North, Central, and South America, as well as in Southern Europe [15, 16]. Most cases reported from Northern Europe were thought to be acquired in southern countries. The highest prevalence is reported in river valleys and humid areas where the environmental conditions are more favourable for the breeding of vectors [16]. However, in some studies from large endemic regions, as in the Canary Islands, the reported seroprevalence in women is 6.7% and in men 6.1%, and the highest seroprevalence in humans corresponded to the areas where the highest canine prevalence is reported [17].

According to the recent review performed by Anvari *et al*. in 2020, the prevalence of *Dirofilaria immitis* in dogs for various continents are as follows: Australia 2.68%, Asia 12.07%, America 11.60%, Europe 10.45% , and Africa 7.57% [18].

The critical factor affecting the prevalence of this infection is the weather, geographical distribution, density and age of dogs, number and distribution of vectors, and diurnal periodicity of microfilaraemia.

The life cycles of all *Dirofilaria* species are similar. The female mosquitoes (*Culex, Aedes, Anopheles, Myzorhynchus*) serve as a vector and as an intermediate host after ingesting a bloody meal from an infected dog [19]. Approximately 60 species of mosquitoes have been shown to support complete larval development [20]. Mansonia, fleas, louses, and ticks have also been vectors. Besides dogs, the definitive hosts may be cats, wolves, coyotes, jackals, muskrats, foxes, bears, otters, beavers, and sea lions.

The adult worms reside in the dog's pulmonary artery and in the right ventricle. The developing embryo

in the pregnant female worm hatches from its eggshell and sheds into the blood as a living first-stage L1 larva (microfilaria) [21]. The circulating microfilariae (L1) are ingested by a female mosquito, where they further develop in Malpighian tubules (MT). For normal development, the optimal environmental temperature should remain above 27°C. Below the threshold temperature of 14°C, the cycle is halted [22].

During the second larva stage (L2), after the first moult, the internal organs of the worms are formed. The second moult occurs at 11–12 days. After 2 moults in MT lasting 10–17 days, L3 develop, resembling miniature adults. L3, also called infective larvae, are injected by mosquito's bite into the skin of the animal or human host. The L3-stage larvae reside in the subcutaneous and fatty tissue with a formed cuticle [23]. The cuticle has important absorptive, secretory, and enzymatic activities.

Three to six days after infection, L3 moults to the fourth larval stage (L4) [24]. The larvae in the dog's subcutaneous tissue mature over the period of 60–120 days until the final moult into the immature L5 occurs [19, 22]. Thereafter, L5 migrate through blood vessels to the right ventricle and pulmonary arteries where the adult worms reach sexual maturity in about 6 months in the animal host [25].

The final size of the female parasite may be 25–30 cm in length (male worms are only 12–16 cm long) and 1–2 mm in diameter [22].

In humans, most larvae do not mature but are arrested and perish in the subcutaneous tissue. Only a few may reach the right heart, fail to achieve sexual maturation, and die. They are swept with the blood into the right pulmonary artery where they obstruct some of the small lung vessels, causing small pulmonary infarctions. These are often seen as solitary subpleural nodules ('coin lesions') with an average diameter of 2 cm (0.7–4.0 cm) [4, 8]. Histology reveals infarcts where the necrosis is surrounded by a fibrotic rim and inflamed granulation tissue. The necrosis is of coagulative type in about two-thirds of cases and caseous in the remaining one-third. Surrounding infiltrates consist of plasma cells, histiocytes, lymphocytes, numerous eosinophils, and scattered Langhans' giant cells with some neutrophils. Charcot-Leyden crystals, microcalcification, and cholesterol clefts are seen in 27%, 22%, and 17% of the nodules, respectively. Adjacent lung parenchyma shows a desquamative interstitial pneumonia-like reaction in 66% and bronchiolitis in 46% of cases. Patchy organizing pneumonia and numerous foci of reactive metaplastic squamous epithelium lining adjacent airways are detected in 34% of cases. Half of the reported cases show focal vasculitis involving muscular arteries [4]. Anatomy of the worm is better appreciated in silver (Gomori methenamine) or PAS-stained histologic sections. Wiegert Van Gieson staining is very helpful in highlighting the worm's structures and the lung vessel emboli [19].

Morphologic characteristics of *D. immitis*, as well a histologic differential diagnosis of other, more common parasitic infestations, are summarized in Table I [26–30].

Awareness of this entity is important because clinical and radiographic presentations of HPD often mimic lung neoplasm, particularly as 70% of patients with dirofilarial lesions are heavy smokers. *Dirofilariasis* should be included in the differential diagnosis of sub-pleural "coin-like" lesions. Besides primary pulmonary carcinoma or haemorrhagic pulmonary infarction, differential diagnosis may also include benign tumours (hamartoma, solitary fibrous tumour of pleura), solitary lung metastasis, necrotizing granuloma (tuberculoma), Wegener's granulomatosis, rheumatoid nodule, fungal lesion, etc. [2].

In multiple pulmonary nodules, diagnostic pitfalls associated with HPD include patchy organizing pneumonia and follicular bronchiolitis. Necrotic tissue may even suggest idiopathic bronchiolitis obliterans organizing pneumonia, extrinsic allergic alveolitis, lymphoma, or metastatic process [4].

Although eosinophilia is commonly associated with parasitic infestations, mild peripheral eosinophilia is found in only 7.5–20% of patients with pulmonary dirofilariasis [1, 3, 31]. Infrequent eosinophilia is due to a very small amount of released antigen [19]. In HPD, surgery can be avoided if serological tests with anti-dirofilarial antibodies are positive and if imaging studies and bronchoscopy rule out malignancy or mycobacterium infection [31].

The serologic tests (complement fixation tests, enzyme-linked immunosorbent assay – ELISA, indirect immunofluorescence, indirect hemagglutination test) have, in general, low specificity [32]. An improved specificity with ELISA has been shown with a fusion protein of 35 kDa and a native polypeptide of 22 kDa (Di22) [33, 34]. Biopsy or surgery is requiring for conclusive diagnosis, but more recently molecular methods based on polymerase chain reaction and sequencing has improved diagnostic accuracy [35, 36].

Conclusions

Humans are dead-end hosts in the life cycle of *Dirofilaria sp.*, and surgical treatment in cases of pulmonary dirofilariasis is unnecessary. The clinicians should be alert to the possibility of dirofilariasis, particularly in individuals who have travelled to endemic areas, as documented in our patients. Because most of the patients are asymptomatic, we believe that HPD is underestimated due to unreported or undiagnosed cases.

The authors declare no conflict of interest.

Table I. Histologic and morphologic characteristics of some more common parasitic infections

Type of parasite		Histological characteristics in lung	Parasite morphology
Protozoa			
Entamoeba histolytica		Pleuritis, empyema, abscess, granulation tissue, mixed inflammation, thickening of the alveolar walls	Trophozoites 10–50 μm, bubbly cytoplasm with engulfed erythrocytes or leukocytes, eccentrically located annular nuclei, small karyosome
Toxoplasma gondii (usually in immunosuppressed, rarely in healthy person)		Lung consolidation, bilateral diffuse or nodular mixed alveolar/interstitial infiltrates with chronic inflammation, interstitial pneumonitis (probably necrotizing and/or DAD), pleural effusions	Intra/extracellular organisms, tachyzoites (oval, round or arc shaped, 2–8 μm) found usually in macrophages, alveolar epithelium, clusters of organisms in pseudocyst
Leishmania spec. (visceral forms considered as opportunistic infection)		Chronic diffuse interstitial pneumonitis, thickening of the alveolar septa with depositing of collagen, macrophages, lymphocytes, plasma cells, septal fibrosis, granulomatous inflammation of bronchial mucosa, pleural effusions, intracellular protozoan in macrophages	Amastigotes almost indistinguishable from trypanosoma's forms
Trypanosoma		Pneumonitis, thickening of the alveolar walls, proliferation of type II pneumocytes, macrophages, mononuclear infiltration, intra-alveolar fibrin, hyaline membranes, erythrocytes, parasites observed in the wall of vessels and bronchia	Flagellate form, 16–22 μm long, with central oval-shaped nucleus, base of flagellum (blepharoplast), in tissue cells transforms into amastigote with rudimentary flagellum and peripheral nucleus, arranged in pseudocysts
Cryptosporidium (usually in immunosuppressed)		Tracheitis with metaplasia, peribronchial glandular hyperplasia, loss of cilia, purulent exudate in the bronchial cavities, thickened intra-alveolar walls with macrophages and lymphocytes, possible progression to DAD	Presence of non-cystic forms sporozoites and merozoites free or in macrophages, oocysts (4–6 μm, spherical shaped)
Plasmodium		Pulmonary oedema, bilateral infiltrates, haemorrhagic areas, capillary congestion, thickening of the alveolar septa, haemozoin (brown pigment) in alveolar macrophages, possible DAD with hyaline membrane or obliterating bronchiolitis and organizing pneumonia	Trophozoites, schizonts and gametocytes – structure depends on the species (e.g. Schuffner dots in infected erythrocytes is specific for P. vivax)
HELMINTHS			
Cestodes	**Ecchinococcus granulosus**	Cysts formation (possible smaller cysts inside larger), with 3 μm thick fibrous capsule, compressed host tissue, ruptured cysts activate granulomatous reaction	Cysts include outer lamellated acellular eosinophilic material, below is germinal layer, ovoid protoscoleces of 100 μm in diameter with 2 internal circular rows of hooklets and 4 suckers, larvae contain calcified bodies – calcareous corpuscles+
	Taenia solium	Cysticerc with thin wall and clear fluid, mixed inflammation with neutrophils, eosinophils and macrophages	Single invaginated scolex with 4 suckers and hooklets, protoscolex attached at an infolded neck region*

Table I. Cont.

TYPE OF PARASITE		HISTOLOGICAL CHARACTERISTICS IN LUNG	PARASITE MORPHOLOGY
Nematodes	**Dirofilaria immitis/repens**	Well circumscribed nodule, with central necrosis (nodular infarct, with the larva usually inside a medium sized vessel), necrotizing or non-necrotizing granulomas, with eosinophils, and vasculitis, surrounded with fibrosis, possible organizing pneumonia, lymphocytes, histiocytes, giant cells	Larvae measure 100–350 μm in diameter with externally ridged of 5–20 μm thick cuticle, with transverse striations, prominent diametrically opposed internal thickening. Abundant somatic muscle projecting into the body cavity. Internal organs: intestine, two uteruses usually without ova in female worm. In the male worm: single reproductive tube without spermatozoa. Distinction between D. repens/immitis: D. immitis has smooth cuticle except on the ventral surface of the male's caudal extremity, D.repens has longitudinal cuticular ridges and transverse striations
	Strongyloides stercoralis	Lung consolidation, haemorrhagic, mixed inflammation with or without granulomas, increased eosinophils, DAD, rarely fibrotic granulomas with interlobar septal fibrosis	Larvae within haemorrhagic alveoli, measure 400–700 μm in length, rows with small nuclei within**
	Ascaris lumbricoides	Lobar consolidation, alveolar haemorrhage, destruction of capillaries and alveolar walls, oedema, epithelial desquamation, chemotaxis of neutrophils and eosinophils	Oval eggs, 50–75 μm long, hatched larvae 0.2 mm long, in the lungs larvae grow to a length of 1–2.4 mm, with visible oesophagus, yellowish-brown granular intestine, genitals
Trematodes	**Paragonimus spec.**	Pleural effusion, pleural thickening (eosinophilic pleuritis), eosinophilic pneumonia, acute and/or chronic bronchiolitis, thin or thick wall cysts adjacent to airways, serpiginous zones necrotizing granulomas with microabscesses adjacent to eggs, vasculitis	Hermaphrodite flukes, spines present on the tegmentum, ventral sucker acetabulum, eggs 90 μm in length, with birefringent wall, shouldered operculum***, eggs empty or contain undifferential larval structures without nuclei
	Schistosoma spec.	Eggs in small arterioles with granulomatous vasculitis and inflammation, marked eosinophils, medial hypertrophy and intimal fibroplasia of pulmonary vessels (due to pulmonary hypertension)	Eggs large 90–180 μm, thin wall, usually without birefringence, with lateral, terminal or rudimentary spines, small nuclei within miracidium

DAD – diffuse alveolar damage
+ calcareous corpuscles – supportive for diagnosis of cestode infection
**Immature scolex within a cysticerc is single and attached to a neck region, while protoscoleces in E. granulosus are smaller, numerous, floating free within a cyst*
***Differential diagnosis for larvae includes Ascaris, Toxocara (eosinophilic abscesses), Wuchereria (eosinophilic alveolitis)*
****Schistosoma eggs do not have operculum, have thinner wall without birefringence, have lateral, terminal, and rudimentary spine*

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