Cryptococcus gattii: An emerging cause of pulmonary nodules

Gary J Dewar MD1, James K Kelly MB ChB2

BACKGROUND: Since the fall of 1999, a new endemic focus of Cryptococcus gattii serotype B infection has emerged on Vancouver Island (Victoria, British Columbia), with infections occurring in both animals and humans. In the human cases, symptoms have manifested as pulmonary nodules, meningitis or both. This organism has added a new nonmalignant cause of pulmonary nodules to the literature, resulting in a change in the management of these nodules by healthcare professionals.

METHODS: A search of the number of cases recorded and treated in hospitals of the Vancouver Island Health Authority, along with a review of the literature regarding this emerging organism, was undertaken. The pathology, epidemiology and clinical course of this previously uncommon fungus were determined, and representative cases were chosen for illustration.

RESULTS: More than 130 cases were recorded in the six-year period from late 1999 to mid-July 2006. The number of cases increased steadily over this period, but appears to be levelling off. Representative cases with medical imaging, along with photos of the pathology, are included. Recommendations for diagnosis, treatment and follow-up are outlined.

CONCLUSIONS: The emergence of cryptococcal lung and central nervous system lesions on Vancouver Island have made it important to include travel or residence of the island as part of the history in patients with pulmonary nodules. A registry of patients from Vancouver Island has been established, and it may be of value to include nonisland patients who are found to be infected with this organism.

Key Words: Benign lesions; Infection; Lung; Pathology

Since the fall of 1999, a new endemic focus of Cryptococcus gattii serotype B (formerly Cryptococcus neoformans var gattii) infection has emerged on Vancouver Island, British Columbia, causing both animal and human disease (some fatal) (1-4). The affected region is along the south-central part of the coast of Vancouver Island. The organism has been cultured from trees and air samples; it appears to grow on the bark of trees, releasing infectious propagules into the air (2). Air concentrations of C. gattii have been measured in the rainy winters (KH Bartlett, personal communication). This new focus of infection may be attributed to a recent recombination event. Unlike most isolates, which are of both alpha and alpha mating types but predominantly sterile, the majority of the Vancouver Island, British Columbia, outbreak strains are exclusively of the alpha mating type and the majority are fertile (4). From the fall of 1999 to the present, more than 130 human cases have been encountered. Most of the patients have been residents of the island, but a few cases have arisen in persons who visited the island only briefly. Pets that visited the island have also become infected. Most patients presented with asymptomatic pulmonary nodules or opacities. The range of cases included pulmonary nodules (single or multiple), meningitis or combined lesions. No cutaneous lesions were recorded. Animals, domestic and wild, terrestrial and aquatic that have been infected include Dall's porpoises, domestic cats, dogs, ferrets and other animals, including exotic species such as a tapir (1,2). Animals presented with head or neck nodules, lymphadenopathy, and nasal or pneumatic signs. The porpoises were washed up on the shoreline dead.

A diagnosis was determined mainly by cytology. Some of the animals were followed with cryptococcal antigen titres during treatment with fluconazole. Unlike the human cases,
some of the animals had predisposing conditions that may have lowered their immunity. Many, but not all, of the animals had travelled to the central island (Parksville-Nanaimo area).

Travellers to the San Joaquin (California, USA) valley may acquire *Coccidioides immitis* lung lesions, and travellers to the St Lawrence River (Massachusetts, USA) and Mississippi River (Minnesota, USA) valleys may acquire similar lesions from *Histoplasma capsulatum* infection. It is now evident that travellers to Vancouver Island may acquire *C. gattii* lung and central nervous system (CNS) lesions. Enquiry regarding travel to or previous residence in these areas is an important part of patient history when evaluating lung lesions.

On Vancouver Island, the sudden emergence of this new organism has added to the differential diagnosis of lung lesions. Formerly, most lesions found on chest x-ray and/or computed tomography (CT) scan in this region, unless obviously a granuloma or hamartoma, were considered malignant unless proven otherwise by fine needle aspiration biopsy, by surgical excision, or by following the clinical course for two years or longer.

The present article outlines representative pulmonary case studies of cryptococcal infection. It also discusses the epidemiology, microbiology and radiological features, in addition to the pathological appearances, of the disease. The accumulated local experience and management of the outbreak are discussed.

### PATIENTS AND METHODS

A retrospective review of patient discharges from acute care hospitals of Vancouver Island from April 1, 1999, to March 31, 2006, was conducted. Initially, only data from the Victoria hospitals (Royal Jubilee Hospital and Victoria General Hospital) of British Columbia were available for the first fiscal year (April 1, 1999 to March 30, 2000). However, when the medical records from all of the island hospitals were consolidated, data were available for the entire geographic area (Vancouver Island). Any discharge with a principal diagnosis of cryptococcal infection was included in the present study (Table 1).

It was apparent that there were more patients being diagnosed each year, but levelling in 2006. With increased awareness of this lesion as a possible differential diagnosis, fewer surgical excisional biopsies were being performed in favour of diagnosis by bronchoscopy or transthoracic fine needle aspiration.

Representative cases outlining the presentation of the disease, particularly with reference to pulmonary medicine, surgery, radiology and pathology practices, have been chosen for illustration.

### Case 1

A 54-year-old man was found to have an asymptomatic opacity in the superior segment of the right lower lobe when he was admitted for an acute myocardial infarction (MI). He presented with mild, atypical cardiac symptoms three days after onset and was not a candidate for thrombolysis or angioplasty. He required a pacemaker for bradycardia. After recovery from the MI, a CT scan was performed, demonstrating a 3 cm lesion in the superior segment of the right lower lobe with no adenopathy (Figure 1). He had a 40 pack-year smoking history. There was no other significant medical history. Flexible bronchoscopy showed no endobronchial lesions, and biopsies and washings were nondiagnostic. CT-guided biopsy was negative and did not provide any other nonmalignant diagnosis. He underwent cardiovascular evaluation preoperatively and was considered to be at reasonable operative risk. Four-and-a-half months after his MI, he underwent an attempted right thoracoscopic wedge resection, which had to be converted to a limited thoracotomy. Excisional wedge biopsy revealed a granuloma, which on permanent sections was found to be a cryptococcal
granuloma. He had an uneventful recovery with no cardiac sequelae, and he was discharged in satisfactory condition. No further treatment was advised other than radiological surveillance.

Case 2
A 62-year-old man presented with right posterior chest pain. Chest x-ray revealed an irregular opacity in the right mid-zone of the lung. He had an 18 pack-year smoking history, but quit 30 years previously. CT scan demonstrated two nodules in the right lung, and possibly a third nodule in the left lung (Figures 2A and 2B). The largest nodule was 1.8 cm in the superior segment of the right lower lobe and was suggestive of malignancy. Tuberculosis (TB) testing was negative, and bronchoscopy revealed no endobronchial lesions. Washings and brushings were negative for malignancy. Cryptococcal serology (antigen latex agglutination test) was nonreactive. He was asymptomatic at the time of surgery. At thoracoscopy, the right lower lobe nodule was completely resected, and the frozen section showed a necrotic granuloma, suspicious for Cryptococcus species. Permanent sections confirmed a cryptococcal lesion. There was no fungal growth from swabs of the lung nodule.

Case 3
A 69-year-old woman presented with a harsh cough and shortness of breath. She had a 35 pack-year smoking history, but quit 20 years previously. She was exposed to TB working as a nurses’ aide. She had a past history of ulcerative colitis, requiring a total colectomy, but was not receiving immunosuppressive therapy at the time of presentation. Chest x-ray demonstrated a lesion in the right lower lung zone, and CT scan revealed a 1.6 cm cavitating lesion in the anterior segment of the right lower lobe (Figure 3). Flexible bronchoscopy and percutaneous needle biopsy were noncontributory. TB skin testing was equivocal. At thoracoscopy, an excisional wedge biopsy revealed cryptococcal organisms on frozen section, and was confirmed on permanent section. There was no fungal growth from swabs of the lesion taken at the time of surgery. No antifungal therapy was instituted but surveillance by periodic chest x-ray was advised.

Case 4
A 65-year-old man was referred with mild recurrent hemoptysis. He was otherwise healthy. He had a 45 pack-year smoking history, but quit 15 years previously. He worked as a painter. Chest x-ray revealed a left hilar opacity, likely in the anterior segment of the left upper lobe (Figure 4A). Flexible bronchoscopy was carried out. No endobronchial lesions were seen. Washings were done, and material was sent for cytology and culture. The patient left for a holiday in Germany the next day against medical advice. A result of cryptococcal infection was reported within a week. In the meantime, the patient had become ill in Germany and was admitted to a German hospital with a diagnosis of meningitis. The results of the bronchoscopy were transmitted to the German physicians, and treatment with fluconazole was instituted. The patient made a full recovery within two weeks and returned to Canada. He was maintained on fluconazole for several months, and the opacity in the left upper lobe was resolving (Figure 4B). At follow-up seven months later, the patient was asymptomatic and his chest x-ray was normal.
petent patients who had travelled to Vancouver Island (15).

Infections at Vancouver Hospital included three immunocom-
pected patients who had travelled to Vancouver Island (15).

More recently, isolated cases have been found in India, Singapore, Greece,
lyptus trees planted in other continents (10). More recently,
in decaying eucalyptus wood and has been grown from euca-
strongly associated with eucalyptus trees and produces disease
var

**Dewar and Kelly**

![Figure 4) Case 4. A Chest x-ray showing a left hilar opacity (arrow). B Chest x-ray after six months of antifungal therapy, showing resolution of the opacity (arrow).**

**DISCUSSION**

Cryptococci are encapsulated basidiomycetous yeasts that are
important opportunistic human pathogens. Transmission is by
exposure to an aerosolized inoculum, the infectious propagule
being either the basidiospore of the perfect (sexual) form (Filobasidella neoformans) or the desiccated yeast (5-7). C neo-
formans is a ubiquitous yeast, whereas C gattii, recently identi-

Cryptococcus organisms have highly characteristic mor-
phology, including a mucicarmine-positive capsule, and are
detected on percutaneous fine needle aspirates, in bronchial
washings or occasionally in transbronchial biopsies. Distinction between C neoformans and C gattii cannot be
made by morphology or by immunohistochemistry, but
depends on serotyping. The excisional lung biopsies con-
tained one or more firm nodules with a whitish or yellow-
brown cut surface (sometimes mucoid). The diagnosis was
often established by a scrape smear or imprint. Histologically,
all of the nodules showed a central necrotic zone (or zones) in
which the ghosted outlines of alveoli were discernible.
Organisms were usually readily visible in the necrotic zone,
and not always numerous. The organisms were usually confined to
the necrotic zone (Figures 5A and 5B). The necrotic zone was
delineated by macrophages and sometimes foamy. Most cases showed a palisade of eosinophilic
macrophages surrounding the necrotic area, and a further thin
layer containing a mixture of lymphocytes, plasma cells and
fibroblasts. Foamy histiocytes were present in 11 of 17 open
lung biopsies. In these samples, the foamy histiocytes were
abundant in five cases (29%), forming collections outside the
granuloma and filling the air spaces.

On frozen section, the abundant foamy macrophages were
misdiagnosed once as clear cell carcinoma, and on other occa-
sions, raised the question of a clear cell carcinoma, particularly
when the necrotic zone was not included in the section. One
case, likely an early or recent infection of C gattii infection,
showed several tiny foci of necrosis and a mixed response of
macrophages, eosinophils, lymphocytes and plasma cells. The
fungi in that case were not localized to the centre of the lesion
but were scattered throughout the macrophages. One case of
C gattii infection occurred coincidentally with a bronchi-
oloalveolar cell carcinoma. Another case had metastatic
colon cancer to the lung with a coincidental cryptococcal
lung lesion. This concurrence makes ongoing monitoring very
important when there are multiple lung opacities on imaging.
Also, if immunosuppressive chemotherapy is given as in the lat-
ter case, then careful surveillance and perhaps antifungal pro-
phylaxis may be required. All excised lesions are cultured to
determine whether there are any viable organisms in the
lesions, but cultures often fail to grow. This information may
help decide whether to offer the patient antifungal therapy

immunocompetent hosts and being somewhat resistant to antifungal therapy (16-19).

The emergence of this outbreak of pulmonary lesions due to
C gattii has caused many health care professionals to revise
their approach to the investigation and management of these
lesions. In Canada, most pulmonary lesions, symptomatic or
not, are considered neoplastic until proven otherwise. Travel
history is important to determine the possibility of coccidiomas
or histoplasmosmas. Now, travel to Vancouver Island, a popular
destination for Canadian, American (approximately 1.2 mil-
ion tourists annually), European and Japanese tourists, will be
important for diagnosing people living elsewhere who develop
single or multiple lung opacities, or cryptococcal meningitis.

Although there are no specific imaging characteristics of
cryptococcal pulmonary nodules, they have been found to vary
in number from one to more than 10. The nodules are usually
6 mm to 20 mm in diameter, and are homogeneous on CT
without low attenuation, calcification and, rarely, cavitation
(20). Serology is of little value.

Cryptococcus organisms have highly characteristic mor-
phology, including a mucicarmine-positive capsule, and are
detected on percutaneous fine needle aspirates, in bronchial
washings or occasionally in transbronchial biopsies. Distinction between C neoformans and C gattii cannot be
made by morphology or by immunohistochemistry, but
depends on serotyping. The excisional lung biopsies con-
tained one or more firm nodules with a whitish or yellow-
brown cut surface (sometimes mucoid). The diagnosis was
often established by a scrape smear or imprint. Histologically,
all of the nodules showed a central necrotic zone (or zones) in
which the ghosted outlines of alveoli were discernible.
Organisms were usually readily visible in the necrotic zone,
and not always numerous. The organisms were usually confined to
the necrotic zone (Figures 5A and 5B). The necrotic zone was
delineated by macrophages and sometimes foamy. Most cases showed a palisade of eosinophilic
macrophages surrounding the necrotic area, and a further thin
layer containing a mixture of lymphocytes, plasma cells and
fibroblasts. Foamy histiocytes were present in 11 of 17 open
lung biopsies. In these samples, the foamy histiocytes were
abundant in five cases (29%), forming collections outside the
granuloma and filling the air spaces.

On frozen section, the abundant foamy macrophages were
misdiagnosed once as clear cell carcinoma, and on other occa-
sions, raised the question of a clear cell carcinoma, particularly
when the necrotic zone was not included in the section. One
case, likely an early or recent infection of C gattii infection,
showed several tiny foci of necrosis and a mixed response of
macrophages, eosinophils, lymphocytes and plasma cells. The
fungi in that case were not localized to the centre of the lesion
but were scattered throughout the macrophages. One case of
C gattii infection occurred coincidentally with a bronchi-
oloalveolar cell carcinoma. Another case had metastatic
colon cancer to the lung with a coincidental cryptococcal
lung lesion. This concurrence makes ongoing monitoring very
important when there are multiple lung opacities on imaging.
Also, if immunosuppressive chemotherapy is given as in the lat-
ter case, then careful surveillance and perhaps antifungal pro-
phylaxis may be required. All excised lesions are cultured to
determine whether there are any viable organisms in the
lesions, but cultures often fail to grow. This information may
help decide whether to offer the patient antifungal therapy

156 Can Respir J Vol 15 No 3 April 2008
REFERENCES


An emerging cause of pulmonary nodules

Figure 5) A Photomicrograph of a necrotic lung nodule containing multiple cryptococci with haloes in the necrotic debris. The granulomatous border of the nodule is visible on the lower left-hand side (hematoxylin and eosin stain). B Photomicrograph of cryptococci within the necrotic debris. Some organisms are round and some are oval. The mucinous capsules are clearly seen in the organisms on the lower right-hand side of the image (hematoxylin and eosin stain, original magnification ×400)

postresection. In the case of a fully resected solitary lesion or asymptomatic multiple nodules where organisms are found but fail to grow, antifungal therapy is generally not recommended. Careful evaluation for any CNS symptoms must be performed, and spinal fluid tested if CNS involvement is suspected. If a lesion is fully resected and the patient is otherwise at no particular risk, then only periodical surveillance is necessary. To date, no cases of recurrent lesions have been reported once successfully treated. Because we are seeing more cases of these lesions, fewer thoracoscopic or open biopsies are required to establish a diagnosis.

The emergence of cryptococcal lung and CNS lesions on Vancouver Island have made it important to include travel to or residence of the island as part of the history when evaluating patients with pulmonary nodules. A registry of patients from Vancouver Island has been established, and it may be of value to include nonisland patients who are found to be infected by this organism.