Invasive aspergillosis complicating treatment with Tyrosine Kinase Inhibitors

SUMMARY Up to 150 words summarising the case presentation and outcome (this will be freely available online)

We describe three cases of pulmonary aspergillosis (PA) in three patients without traditional risk factors for invasive aspergillosis infection, such as prolonged neutropenia or high dose systemic corticosteroid therapy. All three patients developed PA whilst taking tyrosine kinase inhibitors (TKI) and sustained greater clinical improvement once TKI were withdrawn. Our case series supports the theory TKI treatment can increase susceptibility to PA without causing neutropenia. Recognition that TKI treatment may predispose to invasive aspergillosis will allow for rapid recognition of patients of affected patients and more effective management of future cases.

BACKGROUND Why you think this case is important – why did you write it up?

The mitogen-activated protein kinase (MAPK) signalling pathway is involved in the pathophysiology of numerous cancers, including melanoma, leukaemia, pancreatic, colon, lung, biliary tract, salivary gland and thyroid carcinoma.[1,2] As a consequence, tyrosine kinase inhibitors that lead to MAPK pathway inhibition (MAPKi) are increasingly used in the treatment of malignant disease. Treatment with TKI causes a degree of immunosuppression and has been associated with the development of various infections including gastroenteritis, upper respiratory tract infection, acute bronchitis, pneumonia, bursitis, cellulitis and pyelonephritis.[3-5] A randomised double-blind placebo-controlled study of a highly selective p38 MAPK inhibitor demonstrated increased risk of infection compared with placebo (11% vs 5%), particularly serious infections (4.8% vs 0%).[3]

An important infection of severely immunocompromised patients is acute pulmonary aspergillosis (PA), which is most commonly associated with prolonged neutropenia or treatment with high dose systemic corticosteroids.[6,7] More indolent forms of pulmonary aspergillosis are associated with chronic lung disease and milder immunodeficiencies. Here we describe three cases of PA developing in patients receiving TKI treatment, an association that has only recently been reported with ibrutinib.[8-14]

CASE PRESENTATION Presenting features, medical/social/family history

Patient 1
A 63-year-old female was commenced on ibrutinib 420mg OD for chronic B cell lymphocytic leukaemia (CLL). Previous chemotherapy treatment included 2 cycles of fludarabine, cyclophosphamide and rituximab (FCR) over 40 months prior, and 2 cycles of reduced intensity FCR 27 months prior, to starting ibrutinib. She had also received 4 months of Ofatumumab (monoclonal antibody to CD20) as part of a trial, which was stopped 4 months before commencing ibrutinib due to rising white cell count. She required one tapering course of systemic steroids for autoimmune haemolytic anaemia eight months prior to commencing ibrutinib. On day +10 of ibrutinib the patient presented with fever and cough productive of yellow sputum and was treated with seven days of co-amoxiclav and clarithromycin. She re-presented to hospital on day +15 of ibrutinib treatment with persistent fevers and rigors, and new mild left lower limb
pyramidal weakness. There were multiple new densities throughout both lungs on her chest x-ray. Neutrophil count was 1.39. Computed tomography (CT) scanning revealed multiple bilateral pulmonary (Fig. 1a), liver (Fig. 1b) and thyroid nodules, and space occupying lesions of the brain (Fig. 1c). CT guided biopsy of a right lower lobe lung nodule demonstrated fungal hyphae on histopathology and was culture positive for *Aspergillus fumigatus*. Additional liver and skin biopsies demonstrated the presence of *Aspergillus fumigatus* on histopathological analysis, with growth also on fungal culture. Thyroid nodule fine needle aspiration (FNA) showed necrosis and ghosts of fungal hyphae only. Brain biopsy was not performed. Despite treatment with triple antifungal therapy (ambisome, voriconazole and amphotericin B) and ibritinib, the patient developed a new pustular lesion to the left arm. Skin punch biopsy demonstrated necrosis, fungal spores and hyphae suggestive of *Aspergillus* on histopathology, and fungal culture demonstrated *Aspergillus fumigatus*. There was also radiological progression on CT chest. Once ibritinib therapy was stopped no new lesions developed either clinically or radiologically. The patient was discharged home with posaconazole and flucytosine but continued to clinically deteriorate due to aggressive CLL and died on day +267.

**Patient 2**
A 58-year-old female with chronic myeloid leukaemia was treated with a succession of TKI over eight years, initially with imatinib, followed by nilotinib, dasatinib and finally changing to bosutinib 500 mg OD due to intolerance of the previous TKI. She had not received any other chemotherapy agent or systemic corticosteroids and had no history of neutropenia. She presented on day +61 of bosutinib therapy with breathlessness and was treated with a course of amoxicillin with no clinical improvement. The patient re-presented to our hospital on day +89 with fever, cough and dyspnoea. Construction work was being conducted within her home during this time. Chest x-ray demonstrated bilateral patchy areas of consolidation. Neutrophil count was 7.37. CT scan demonstrated multiple bilateral pulmonary macronodules with surrounding halos characteristic of PA (Fig. 2a). Blood and sputum cultures were negative. Serum galactomannan was not performed. The patient was too hypoxic to have a bronchoscopy. She was commenced empirically on ambisome, imipenem and amikacin, and the bosutinib was stopped. She made steady clinical and radiological improvement, and the antibiotics were stopped after two weeks. After 12 days the ambisome was switched to oral voriconazole for two months. She made a full clinical recovery, and repeat CT scanning showed resolution of the macronodules with residual focal scarring (Fig. 2b). Although Patient 2 lacked microbiological evidence of *Aspergillus* infection, the CT changes and steady clinical and radiological improvement with anti-fungal treatment was highly suggestive of PA rather than other infective diagnoses.

**Patient 3**
A 72-year-old male underwent a gastrectomy for a gastrointestinal stromal tumour of the stomach and was subsequently commenced on imatinib 200 mg OD, increasing to 300 mg, then 400 mg after six months. After 12 months of imatinib treatment, routine interval CT imaging revealed a new small cavitating left apical lung lesion at the site of a previously stable solid nodule (Fig. 3a). Although the patient remained asymptomatic, subsequent imaging over the next twelve months showed slowly progressive changes with the development of a new cavitating left upper lobe lesion and patchy nodular infiltrates. The patient was never neutropenic. Bronchoscopy was performed and bronchoalveolar lavage fluid from the left upper lobe contained fungal hyphae when examined by microscopy, and was culture positive for *Aspergillus fumigatus* and *Aspergillus flavus*. Bacterial and mycobacterial cultures were negative. He was treated initially with voriconazole 200 mg BD, which was stopped due to rash. Although voriconazole may increase plasma levels of TKIs, the dose of imatinib did not have to be adjusted after the introduction of voriconazole, with the measured voriconazole levels being within target therapeutic range. Repeat CT scan demonstrated progressive changes and IV caspofungin was commenced for 40 days. After four months, CT imaging demonstrated further progression and the patient was commenced on itraconazole, which was stopped within 2-3 weeks as he developed tremor and a flu-like reaction. At this time imatinib was stopped, and subsequently there was radiological (Fig. 3b) and microbiological improvement, with

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negative Aspergillus culture on bronchoalveolar lavage, despite the patient not receiving continuing antifungal treatment.

INVESTIGATIONS *If relevant*

DIFFERENTIAL DIAGNOSIS *If relevant*

TREATMENT *If relevant*

OUTCOME AND FOLLOW-UP

(Please note that investigations, treatment and outcomes have been included within each case above for clarity and ease of reading. This is particularly necessary for the cases where further investigations and treatments occurred and I would be concerned that to split the cases up into sections would result in circular and difficult to read manuscript. I hope that is acceptable.)

DISCUSSION *Include a very brief review of similar published cases*

Here we describe three cases of PA which did not at the time of developing the infection have the conventional high-risk factors for this infection (eg neutropenia, high dose systemic corticosteroids),[6,7] but were taking TKI. Patient 1 had biopsy-proven disseminated PA. Patient 2’s CT changes and steady clinical and radiological improvement with anti-fungal treatment was highly suggestive of PA rather than other infective diagnoses. Patient 3 had radiological and microbiological evidence of slowly progressive PA. Withdrawal of TKI treatment was associated with no further progression of disseminated aspergillosis for Patient 1, despite prior progression on antifungals. Withdrawal of TKI treatment and concurrent antifungal treatment was associated with clinical and radiological improvement in Patient 2. Patient 3 demonstrated radiological and microbiological improvement on withdrawal of TKI, despite suboptimal antifungal therapy due to drug intolerance. These cases suggest that despite not causing neutropenia, treatment with TKI does increase susceptibility to Aspergillus infection. Our case series also suggests withdrawal of TKI promotes clinical recovery and should be considered in patients with significant evidence of active Aspergillus infection. Azole sensitivity testing is not routinely performed at our hospital but would have been important if any of our cases had exhibited poor clinical response to single agent azole therapy.

If IPA is suspected clinically, early diagnostic tests should include blood and sputum cultures, serum galactomannan and early CT chest. Bronchoscopy with bronchoalveolar lavage, or diagnostic tissue sampling with transbrachial or transthoracic biopsies, are useful to obtain microbiological diagnosis, but may not be suitable for all patients.

A recent retrospective study shows increased incidence of invasive fungal infection in CLL patients taking ibrutinib.[8] Invasive aspergillosis accounted for 27/33 cases with a 11/27 demonstrating cerebral involvement. Another retrospective study has shown 37.2% of patients receiving ibrutinib for lymphoid cancer developed invasive fungal infection.[9] Multiple case reports also support an association of invasive aspergillosis with ibrutinib therapy,[10-14] with several case reports suggesting an association with cryptococcal infection.[14-20] Mucormycosis and Pneumocystis jirovecii with ibrutinib therapy have also been described.[21-23]
There is considerably less data to support an association between either imatinib or bosutinib treatment with invasive fungal or *Aspergillus* infection. There is one report of early-onset mucormycosis in a patient with acute lymphoblastic leukaemia receiving imatinib alongside other immunosuppressive treatment,[24] and one case of *Candida krusei* and *Candida glabrata* pneumonia in a patient receiving imatinib only for chronic myeloid leukaemia.[25]

Recently, Herbst *et al* described a macrophage cell signaling pathway (TLR9-BTK-calcineurin-NFAT) that requires activation of Bruton’s Tyrosine Kinase (BTK). This macrophage signalling pathway is critically important for rapid recruitment of neutrophils. A deficient early chemokine response leads to impaired neutrophil recruitment and reduced immunity against *Aspergillus* infection in zebrafish.[26] BTK’s importance in innate immune system’s response to fungal infection has also recently been demonstrated in murine models,[27] and has also been implicated in immune responses to *Candida* infection.[28] Ibrutinib, imatinib and bosutinib inhibit BTK, and these findings provide a potential mechanism by which TKI could predispose to PA in patients with a normal circulating neutrophil count.

Recognition that TKI, may predispose to invasive aspergillosis, and potentially other fungal infections, such as cryptococcosis and mucor,[14–21] will allow rapid recognition of affected patients and more effective management of future cases.

**LEARNING POINTS/TAKE HOME MESSAGES 3 to 5 bullet points – this is a required field**

- Long-term steroids and prolonged neutropenia are considered typical risk factors for developing invasive aspergillosis infection
- Treatment with mitogen-activated protein kinase inhibitors may also be a risk factor for invasive aspergillosis
- A high index of suspicion for patients with fever, cough and imaging possibly consistent with invasive aspergillosis in patients taking TKI is recommended, with early commencement of anti-fungal therapy
- Stopping TKI treatment whilst treating for invasive aspergillosis may aid recovery
- A potential mechanism for TKI predisposing to invasive aspergillosis with a normal circulating neutrophil is through inhibition of macrophage cell signalling pathways that are critically important for rapid recruitment of neutrophils against *Aspergillus*

**REFERENCES Vancouver style (Was the patient involved in a clinical trial? Please reference related articles)**

1. Xing L. Clinical candidates of small molecule p38 MAPK inhibitors for inflammatory diseases. MAP Kinase. 2016 Jan 19;4(1).


FIGURE/VIDEO CAPTIONS figures should NOT be embedded in this document
| Figure 1a: Bilateral nodular consolidation on CT chest |
| Figure 1b: Liver nodule on CT imaging of the upper abdomen |
| Figure 1c: Space-occupying lesions demonstrated on MRI brain |
| Figure 2a: Bilateral macronodules with surrounding halos on CT chest |
| Figure 2b: Resolution of macronodules with residual focal scarring on repeat CT chest |
| Figure 3a: Left upper lobe cavitating lesion on CT chest |
| Figure 3b: Filling in of left upper lobe cavity on repeat CT chest |

**PATIENT’S PERSPECTIVE** Optional but strongly encouraged – this has to be written by the patient or next of kin

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