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Epidemiology and clinical characteristics of invasive mould infections: A multicenter, retrospective analysis in five Asian countries

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Key points: Among invasive mould infections (IMIs), invasive aspergillosis is the commonest IMI in Asia. Steroid use and neutropenia are important risk factors, but diabetes is a common underlying condition, particularly in patients without history of steroid exposure or neutropenia.

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Abstract

Formal, large-scale, multicenter studies of invasive mould infection (IMI) in Asia are rare. This 1-year, retrospective study was designed to assess the incidence and clinical determinants of IMI in centers in five countries (Thailand, Taiwan, Singapore, China, India). Patients treated in a single year (2012) were identified through discharge diagnoses, microbiology, and histopathology logs, and entered based on published definitions of IMI. A total of 155 cases were included (median age 54 years; 47.7% male). Of these, 47.7% had proven disease; the remainder had probable IMI. The most frequent host factors were prolonged steroid use (39.4%) and recent neutropenia (38.7%). Common underlying conditions included diabetes mellitus (DM; 30.9%), acute myeloid leukemia (19.4%), and rheumatologic conditions (11.6%). DM was more common in patients with no recent history of neutropenia or prolonged steroid use ($P = .006$). The lung was the most frequently involved site (78.7%), demonstrating a range of features on computed tomography (CT). *Aspergillus* was the most common mould cultured (71.6%), primarily *A. fumigatus* and *A. flavus*, although proportions varied in different centers. The most often used antifungal for empiric therapy was conventional amphotericin. Ninety-day

mortality was 32.9%. This is the first multicenter Asian study of IMI not limited to specific patient groups or diagnostic methods. It suggests that DM and rheumatologic conditions be considered as risk factors for IMI and demonstrates that IMI should not be ruled out in patients whose chest features on CT do not fit the conventional criteria.

Key words: invasive mould disease, invasive aspergillosis, diabetes mellitus.

Introduction

There is increasing international awareness of invasive mould infections (IMI). This is related in part to the explosion in the number of immunocompromised persons, attributed to successes in transplantation and chemotherapy. In addition, the antemortem diagnosis of mould infections has improved, at least in part, after the introduction of new techniques such as the galactomannan (GM) assay, the beta-D-glucan (BDG) assay, and various polymerase chain reaction (PCR) assays.[1] The diagnosis of IMI in immunocompromised persons has, naturally, received the greatest attention. In recent years, international consensus guidelines on the diagnosis of IMI have been developed and refined, and their use has become standard in the literature.[2–4]

The treatment of mould infections has also been transformed by the advent of new antifungal agents such as new triazoles, echinocandins and lipid preparations of amphotericin B. Investigators in the field have reported a decline in the mortality associated with IMI and have attributed it to the use of these newer agents.[5,6]

It is noteworthy that the studies that formed the basis for these comments have come almost exclusively from American and European centers. From epidemiologic studies, to studies testing new diagnostic kits, to drug trials, to algorithmic approaches to IMI—Western centers have dominated the literature.

In comparison, data from Asian countries are scarce, although individual centers have reported high incidence with varied epidemiology. This has been highlighted by regional investigators.[7] Transnational papers are rare, and there is no systematic, intercountry study of the epidemiology of IMIs in Asia. No paper has attempted to provide an overview of IMIs from the clinician's perspective. A comprehensive Japanese report based on autopsy data reported an increasing rate of *Aspergillus* infections but covered all types of fungal infections including yeasts and by its very nature was limited only to autopsy material.[8] An Indian report covered invasive pulmonary aspergillosis over a 12-year period but was also an autopsy series and was based on only 39 patients from one center.[9] A recent, large, single-center Taiwanese study of invasive aspergillosis (IA) noted a significant increase in incidence between 2000 and 2009, but positive cultures were used to identify cases, and hence the report is likely incomplete because it did not include culture-negative cases. [10] A recent Thai and Bahraini series on IA also provide snapshots of the problem at single tertiary centers.[11,12] Finally, an international, prospective study of invasive fungal infections found that *Aspergillus* spp. were associated with the majority of cases and had an overall 30-day mortality of 22.1%.[13] However, this study was limited to patients with hematologic malignancies.

Hence, to date, there has been no large-scale, systematic, international study of the epidemiology of IMIs in Asia. The present multicenter, retrospective study of IMIs at five centers in five countries across Asia was therefore initiated, under the auspices of the Asia Fungal Working Group (a working group of the International Society for Human and Animal Mycology). The primary objective was to determine the incidence and clinical determinants of IMIs in an Asian context. Secondary objectives were to determine the demographics and risk factors for IMI; the types of moulds responsible for IMIs; physician practices in the management of patients with IMIs; and patient outcomes.

Methods

Study design

This was a 1-year, retrospective study of IMIs at five centers in five Asian countries, one each from Bangkok (Thailand), Beijing (China), Chandigarh (India), Singapore, and Taipei (Taiwan). All participating sites were required to meet the following criteria: maintain International Classification of Diseases (ICD) coding and data on the total number of discharges and deaths; manage hematology and transplant patients; perform high-resolution computed tomography (CT) scans; have a microbiology/mycology laboratory that performs isolation and identification of fungi to at least genus level; and have histopathology facilities.

Only diagnoses of an IMI made between 1 January and 31 December 2012 were considered. Ethics approval for the study was obtained from the Institutional Review Board of each site.

Initial patient screening

Potentially eligible patients came from three possible sources: discharge/death diagnosis, microbiology/mycology laboratory records, and histopathology laboratory records.

With regard to discharge/death diagnoses, all cases that carried an IMI as a discharge diagnosis were assessed. One of the following ICD-9 codes was required for eligibility within the study: 117.3 (Aspergillosis); 117.6 (Allescheriosis); 117.7 (Zygomycosis or Mucormycosis); 117.8 (Phaeohyphomycosis, i.e., infection with dematiaceous fungi); 117.9 (other and unspecified mycosis); and 118 (opportunistic fungi, i.e., infection of skin, subcutaneous tissues, and/or organs by a wide variety of fungi generally considered to be pathogenic to compromised hosts only, e.g., infection by species of *Alternaria*, *Drechslera*, *Fusarium*).

For microbiology/mycology data as a source, appropriate laboratory records were reviewed. The case files of patients with evidence of mould infection (e.g., hyphae seen on smears, positive culture for a mould) were then assessed for eligibility within the study.

For histopathology, case records of patients with histology/cytology showing septate hyphae invading tissue were reviewed to assess eligibility within the study.

Study subjects

The electronic and paper records of potentially eligible patients identified by one of the three methods described above were assessed against the study inclusion criterion, which required diagnosis of a proven or probable IMI, based on published definitions.[2–4]

Proven IMI was based on the definition from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) guidelines: cases were considered proven if histopathology, cytology, or direct microscopy demonstrated septate hyphae invading tissue or in aspirate from sterile sites, or if there was a positive culture from a sterile site.[2]

Probable IMI cases were included if they met one of three sets of criteria, selected on the basis of the host: immunocompromised patients fulfilling the EORTC/MSG criteria for probable IMI;[2] patients in an intensive care unit meeting the criteria for a probable case based on the guidelines from Blot et al.;[3] and patients with chronic obstructive pulmonary disease (COPD) fulfilling the criteria for a probable case set out by Bulpa and coworkers.[4]

For patients with sinusitis from the Indian center, a modification of the EORTC/MSG guidelines was applied. Diabetes mellitus (DM) was accepted as a host criterion, and the clinical criteria were modified such that pain alone was inadequate; there had to be a black eschar or radiologic evidence of extension across bone. EORTC/MSG mycologic criteria had to be met.

Patients were excluded if they had endemic mycoses (e.g., histoplasmosis), sporotrichosis, penicilliosis, yeast infections, allergic fungal diseases like allergic bronchopulmonary aspergillosis, or infection limited to the skin or eye.

Assessments

The case records of eligible patients were reviewed and the following data collected: demographic information; host factors (defined according to EORTC/MSG criteria) and underlying conditions that may have predisposed them to acquiring an IMI; sites of involvement; clinical manifestations; radiologic features (in patients with lung involvement); laboratory investigations, including fungal microscopy, cytologic examination, fungal cultures, and serum galactomannan (GM); empiric/prophylactic and definitive treatments (empiric use of antibiotics/antifungals was defined as administration before a microbiologic result was known); and 90-day mortality.

Assessment was also made of risk factors for 90-day mortality, and of the association between two specific host factors (recent history of neutropenia and prolonged use of steroids) and other patient-related variables: age; DM; fever; lung involvement; skin/subcutaneous involvement; disseminated disease (defined as involvement at two or more noncontiguous sites, with involvement of lung and sinus not taken as disseminated but classified as a separate category[14]); surgery; and survival at discharge.

For the purposes of this study, 'any lung involvement' was defined as lung infection alone, as part of a lung plus sinus syndrome, or as part of disseminated disease. The term 'sinus-related' refers to cases with sinusitis only, sino-orbital disease, sino-orbito-cerebral disease, or sino-cerebral disease. Cases of aspergillosis included those that were culture-positive for *Aspergillus*, as well as those positive for GM (unless they grew a non-*Aspergillus* mould).

Appropriate data were recorded electronically and sent to the collating center in Singapore, where they were reviewed for completeness and internal consistency and subsequently analyzed. Whenever doubts existed (e.g., discrepant information), clarification was sought from individual sites.

Statistical analyses

Data were extracted for statistical analysis using SAS Version 9.3 for Windows (SAS, Inc. Cary, NC USA). Data are

Table 1. IMI cases by country.

Country	*n* (%)	IMI incidence per 1000 patient days
Singapore	23 (14.8)	0.0444
Thailand	66 (42.6)	0.2621
China	14 (9.0)	0.0242
India	12 (7.7)	0.0308
Taiwan	40 (25.8)	0.0531

reported using descriptive statistics, including median/mean and range for continuous variables and frequency and percentage for categorical variables.

The association between recent history of neutropenia/prolonged use of steroids and other patient-related variables was analyzed using the two-sample *t*-test for continuous variables and the χ^2/Fisher exact test for categorical variables.

Multiple logistic regression analysis was also carried out to determine risk factors for death 90 days after diagnosis. Variables with *P*-value < 0.20 in a univariate analysis, as well as clinically significant variables that did not reach statistical significance, were incorporated in the initial model. Multiple logistic regression models were then built, yielding the best prediction using a stepwise selection approach.

Statistical significance was set at $P < .05$.

Results

Aggregated results across all five centers are presented. Additional data are available in Supplementary Material 1, and data from individual centers are provided in Supplementary Material 2.

Patient characteristics and demographics

A total of 185 case report forms were received. Of these, 30 were rejected because they did not meet the inclusion/exclusion criteria. Hence, 155 cases were included in the present analysis. 66 came from Thailand, 40 from Taiwan, 23 from Singapore, 14 from China, and 12 from India (Table 1). IMI incidence rates varied between 0.0242 and 0.2631 per thousand patient-days (Table 1).

Among the 155 included cases, the median age was 54 years (range, 4–84 years) and 74 (47.7%) were male. A total of 74 (47.7%) had proven IMI. The remaining 81 patients (52.3%) had probable IMI: 74 based on EORTC/MSG criteria (including four with sinusitis from India, using modified criteria), five by the Blot criteria, and two by the Bulpa criteria (Supplementary Material 2).

Table 2. Host factors and underlying conditions.

Host factors	***n*** **(%)**[a]
Prolonged use of corticosteroids[b]	61 (39.4)
Recent history of neutropenia[c]	60 (38.7)
Treatment with T-cell immunosuppressant[d]	45 (29.0)
Receipt of allogeneic HSCT	17 (11.0)
Treatment with specific monoclonal antibodies[e]	6 (3.9)
Receipt of solid organ transplant	5 (3.2)
Underlying conditions	***n*** **(%)**[a]
Hematologic malignancies	
AML	30 (19.4)
ALL	16 (10.3)
Lymphoma	13 (8.4)
Other hematologic malignancy/leukemia	7 (4.5)
CML	
CLL	
Multiple myeloma	
Post-allogeneic HSCT with no GVHD	9 (5.8)
Post-allogeneic HSCT with GVHD	7 (4.5)
Severe aplastic anemia	4 (2.6)
Receipt of autologous HSCT	1 (0.6)
Other conditions	
Diabetes mellitus	48 (30.9)
Rheumatologic condition	18 (11.6)
Other condition on steroids	14 (9.0)
Liver disease	7 (4.5)
COPD	6 (3.9)
Solid organ malignancy	4 (2.6)
HIV/AIDS	1 (0.6)

[a]Expressed as percentage of total number of subjects ($n = 155$); total exceeds 155 as some subjects had > 1 host factor or clinical condition present.
[b]At a mean minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks.[7]
[c]$< 0.5 \times 10^9$ neutrophils/L for > 10 days temporally related to onset of fungal disease.[7]
[d]Including cyclosporine, occurring in the past 90 days.[7]
[e]In the past 90 days, e.g., alemtuzumab or rituximab or other.[7]
AIDS, acquired immunodeficiency syndrome; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; COPD, chronic obstructive pulmonary disease; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplant; HIV, human immunodeficiency virus.

Host factors and underlying conditions that may have predisposed patients to acquiring an IMI are provided in Table 2. The most common host factors were prolonged use of corticosteroids ($n = 61$; 39.4%) and a recent history of neutropenia ($n = 60$; 38.7%). Among the underlying conditions, DM was the most common ($n = 48$; 30.9%), followed by acute myeloid leukemia (AML; $n = 30$; 19.4%) and rheumatologic conditions ($n = 18$; 11.6%).

The 48 DM cases were further analyzed. Twenty-eight patients had other underlying conditions; in the remaining 20 cases (12.9% of the entire study population), DM was

Table 3. Diabetes cases.

Diabetes cases with/without underlying conditions	Total $n = 48$
Diabetes only	20
Diabetes with coexisting conditions	28
AML	7
ALL	4
Lymphoma	4
Post-allogeneic HSCT no GVHD	1
Post-allogeneic HSCT with GVHD	2
Solid organ malignancy	2
COPD	3
Chronic liver disease	1
Fulminant liver disease	1
Rheumatologic condition	3
Other conditions on steroids	7
Diabetes as sole underlying condition	**Total $n = 20$**
Probable	6
Modified diabetes criteria in sino-orbital	4
Blot eligible[a]	2
Proven	14
Syndrome	
Pulmonary	5
Sinus related	8
Soft tissue-related	1
Lung and sinus-related	3
Others	3

[a]In the Blot criteria, clinical factors are not needed.
ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; COPD, chronic obstructive pulmonary disease; GVHD, graft versus host disease; HSCT, hematopoietic stem cell transplant.

the only underlying condition, of which 14 had a proven and six had a probable IMI (Table 3).

Clinical presentation

Classification of IMIs according to the sites of involvement are provided in Table 4. The lung was the most commonly involved site, affecting 122 patients (78.7%), following by sinus-related cases ($n = 17$; 11.0%). Pulmonary involvement was the sole manifestation of IMI in 95 cases (61.3% of the entire study population).

Clinical manifestation data were also collected (Table 5). Fever was the most frequent ($n = 107$; 69.0%), and various respiratory symptoms were also common, particularly cough ($n = 78$; 50.3%) and breathlessness ($n = 77$; 49.7%). Pleuritic chest pain was recorded in 25 patients (16.1%), all of whom had aspergillosis.

Table 4. Frequency of IMI by site of involvement.

Sites of involvement	n (%)[a]
Lung only	95 (61.3)
Sinus-related	17 (11.0)
Sinusitis only	5
Sino-orbital	8
Sino-orbital-cerebro	3
Sino-cerebro	1
Soft tissue-related[b]	8 (5.2)
Other[c]	7 (4.5)
Disseminated (≥ 2 noncontiguous sites)[d]	15 (9.7)
Lung and sinus-related	13 (8.4)
Any lung	122 (78.7)

[a]Expressed as percentage of total number of subjects ($n = 155$).
[b]In one case, bone was also involved.
[c]Brain ($n = 1$), osteoarticular ($n = 1$), kidney ($n = 1$), peritoneal dialysis fluid ($n = 3$), ear ($n = 1$).
[d]Cardiac + brain ($n = 1$), cardiac + lung + sinus ($n = 1$), lung + brain ($n = 4$), lung + liver ($n = 1$), lung + liver + kidney ($n = 1$), lung + sinus + brain ($n = 1$), lung + sinus + joint ($n = 1$), lung + skin ($n = 4$), lung + sinus + appendix (= 1).
IMI, invasive mould infection.

Radiologic and laboratory investigations

Radiologic features of the patients with lung involvement ($n = 122$) are shown in Table 6. In almost half of cases, a CT scan of the thorax showed a mix of features ($n = 57$; 46.7%). The halo sign was seen in only 5 patients (4.1%). On chest X-ray, consolidation ($n = 37$; 23.9%) was slightly more common than nodules ($n = 34$; 21.9%).

Ninety-five patients (61.3%) had specimens submitted for fungal microscopy; of these, 51 (53.7%) were positive for hyphae (Supplementary Material 1). In addition, 92 patients had specimens submitted for cytologic examination, and 46 (50.0%) were positive for hyphae (Supplementary Material 1). Sinus aspirates tended to have the highest yield for both types of smears.

With regard to fungal cultures, these were performed for 125 subjects (80.6%), with 88 (70.4% of 125) recording positive results (Table 7). Bronchoalveolar lavage fluid was the most commonly cultured site ($n = 65$), and contributed to 33 of the 88 positive results (37.5%). A lung biopsy was performed in 21 cases and yielded mould by culture in 14 instances (66.7%). All 15 patients with sinusitis had a positive culture for a mould from sinus aspirate (Table 7).

The most commonly cultured mould was *Aspergillus*, accounting for 63 (71.6%) of all 88 positive cultures (Table 7). *A. fumigatus* was the leading *Aspergillus* species. Mucormycetes were responsible for nine infections (10.2%).

The distribution of mould species by site of involvement is shown in Figure 1. *Aspergillus* was the dominant species in pulmonary, sinusitis, disseminated & other cases. By contrast, soft tissue infections were caused by a variety of other

Table 5. Clinical manifestations.

Clinical symptoms related to IMI	*n* (%)[a]
General symptoms	
Fever	107 (69.0)
Fever refractory to ≥ 3 days of broad-spectrum antibiotic therapy	81 (52.3)
Recrudescent fever	38 (24.5)
Respiratory manifestations	
Cough	78 (50.3)
Breathlessness	77 (49.7)
Dyspnea	46 (29.7)
Pleuritic chest pain	25 (16.1)
Hemoptysis	20 (12.9)
Worsening respiratory insufficiency	19 (12.3)
Pleural rub	2 (1.3)
Sinus-associated symptoms/signs	
Facial/sinonasal pain	21 (13.5)
Extension from paranasal sinus across bony barrier, including into the orbit	15 (9.7)
Vision loss	15 (9.7)
Nasal ulcer with eschar	8 (5.2)
Cranial nerve palsy	3 (1.9)
Other	
Headache	30 (19.4)
Confusion	14 (9.0)
Skin nodule	13 (8.4)
Abdominal pain	12 (7.7)
Swollen joint	6 (3.9)
Seizure	4 (2.6)
Back pain	3 (1.9)
Oliguria/anuria	1 (0.6)
Flank pain	1 (0.6)

[a]Expressed as percentage of total number of subjects (n = 155); total exceeds 155 as some subjects had > 1 clinical manifestation.
IMI, invasive mould infection.

Table 6. Imaging results in patients with lung involvement.

CT chest signs	*n* (%)[a]
Mix of features	57 (46.7)
Nodule without halo	43 (35.2)
Cavity	33 (27.0]
Consolidation	10 (8.2)
Halo sign present	5 (4.1)
Air crescent sign	4 (3.3)
With ground glass changes	2 (1.6)
Chest X-ray signs	***n*** **(%)**
Consolidation	37 (23.9)
Nodule(s)	34 (21.9)
Combination of features	24 (15.5)
Pulmonary edema/PCP-like picture	9 (5.8)
On CT and X-ray	***n*** **(%)**
Pleural effusion	39 (25.2)
CT sinus	***n*** **(%)**
Sinus imaged	36/155 (23.2)
Only sinusitis[b]	14/36 (38.9)
Bone destruction[b]	2/36 (5.6)
Orbital involvemen[tb]	7/36 (19.4)
Cranial fossa/cerebral involvement[b]	5/36 (13.9)

[a]The percentages are based upon number of cases with any lung involvement ($n = 122$).
[b]The denominator includes cases with sinus-related disease ($n = 36$).
CT, computed tomography; PCP, *Pneumocystis carinii pneumonia*.

moulds. Skin and soft-tissue involvement was a significant predictor of a non-*Aspergillus* mould ($P < .001$) (Supplementary Material 1).

A total of 111 patients were tested for serum GM, and this was positive in 75 cases (67.6%). However, in two cases, it was probably a false-positive, as cultures grew mucormycetes. Of the positive serum GM results, 52% came from neutropenic patients.

Treatment and outcomes

Empiric antifungal treatment was given to 94 patients (60.6%), and 98 (63.2%) received definitive treatment consisting only of antifungal agents (Table 8). The most commonly used antifungal for empirical therapy was amphotericin B deoxycholate (AMB), and for targeted therapy was voriconazole (VCZ).

Ninety-day mortality among the 155 patients in this study was 32.9%. By univariate analysis, risk factors for 90-day mortality included older age, disseminated disease, prolonged use of steroids, exposure to a T-cell suppressant, a rheumatologic condition, higher serum GM, empiric treatment with fluconazole, and short treatment duration. Using multiple logistic regression analysis, disseminated disease, a rheumatologic condition, and higher serum GM levels were found to be potential predictors of mortality (Supplementary Material 1).

Association between key host factors and other variables

An analysis was performed to examine the association between the two most common host factors (prolonged use of corticosteroids and a recent history of neutropenia) and various patient variables, for example, demographics and underlying conditions (Table 9).

A recent history of neutropenia was found to be significantly associated with younger age, absence of DM, fever, and lung involvement. Prolonged use of steroids was significantly associated with lung involvement, absence of

Table 7. Mould culture.

Mould culture	***n*** **(%)**
Cases with culture done	125/155 (80.6)
Cases with mould cultured	88/125 (70.4)[a]
Mould cultured by site	**Mould cultured/ culture done (%)[a]**
Bronchial wash	33 /65 (50.8)
Tissue biopsy, other	30 /35 (85.7)
Sputum	16 /32 (50.0)
Sinus aspirate	15 /15 (100.0)
Tissue biopsy, lung	14 /21 (66.7)
Endotracheal tube aspirate	3 /5 (60.0)
Tissue biopsy, skin	3 /7 (42.9)
Type of mould cultured	***n*** **(%)[b]**
Aspergillus	63/88 (71.6)
A. fumigatus	31
A. flavus	24
A. niger	2
A. sydowii	1
A. nidulans	1
Aspergillus spp.	4
Mucormycetes	9/88 (10.2)
Rhizopus arrhizus	2
Rhizopus spp.	1
Rhizomucor variabilis	1
Rhizomucor spp.	1
Cunninghamella spp.	2
Mucor spp.	2
Fusarium	2/88 (2.3)
Fusarium spp.	2
Other	7/88 (8.0)
Phaecremonium spp.	1
Pyrenochaeta romerei	1
Schizophylum commune	1
Chrysonilia sitophila	1
Dematiaceous mould	2
Exophiala spp.	1
Mixed cultures	7/88 (8.0)
A. fumigatus and *A. flavus*	5
Rhizopus arrhizus and *A. flavus*	1
Colletotrichum coccodes and *Cunninghamella* spp.	1

[a]Expressed as a percentage of the number of cultures submitted.
[b]Among 88 cases with positive cultures, the mould identity and numbers are provided, including subjects with more than one mould species cultured.

surgery, and death before discharge. When these two host factors were combined, the presence of either recent neutropenia or prolonged use of steroids correlated with a number of variables: absence of DM, fever, lung involvement, absence of surgery, and death before discharge (Table 9).

Discussion

To the best of our knowledge, this 1-year, retrospective study is the first multicenter Asian report on IMI that is not limited to specific patient groups or diagnostic methods. IMI incidence rates varied between 0.0242 and 0.2631 per thousand patient-days across the five centers. However, the actual burden of IMI may be higher. Thirty out of 185 case report forms submitted (16.2%) were excluded—around a third because they did not meet the EORTC/MSG criteria. There was a particularly high rejection rate of submissions from India, possibly due to high levels of familiarity with the clinical presentation of IMI, which may have led to necessary interventions being made with less effort devoted to proving the diagnosis using published criteria. This contrasts with Singapore, where the proportion of proven cases was the highest. The need to prove the diagnosis may reflect Singapore's more litigious society, a more questioning populace,[15] and a payment system in which patients are effectively using 'their own' money.

The difficulty of fulfilling the inclusion criteria for clinical studies of this type has been highlighted previously, and it has been suggested that the EORTC/MSG criteria are too restrictive.[16,17] In the present work, some adjustments were made. In particular, recognizing that sinocranial mycosis is well documented in India; the EORTC/MSG criteria were modified to include DM as the sole host criterion. However, to be accepted, cases still had to have bone erosion or extension to an adjacent site.[18–20] This need to modify current criteria to allow the inclusion of patients with clinically obvious fungal rhinosinusitis highlights an important gap. Strictly speaking, published criteria in this area are merely a means of categorizing fungal rhinosinusitis and can only be applied after histopathology is available. [21,22] Hence, there is a need for consensus standards for the clinical diagnosis of IMI of the sinuses, perhaps modeled on the EORTC/MSG criteria.

A key finding from the present work was that DM was an important underlying condition, found in around a third of the subjects in this study. Previous reports have also noted this link.[12,23] For example, patients with diabetic ketoacidosis may be particularly susceptible to rhinocerebral mucormycosis.[24] Furthermore, in a recent Indian series, uncontrolled DM was a risk factor for this condition, even in the absence of ketoacidosis.[25] The large number of cases from India has been attributed to the high prevalence of DM and a local love of sweets.[25] However, DM has also been recognized as a risk factor for IMI outside India.[26–28] Indeed, a Thai IA series found DM as an underlying condition in 17% of cases. [11]

Of the 48 patients with DM in this study, almost half (20) had no other underlying condition. This aligns with a small Thai study, which noted DM in all patients with sinus-related IMI and found that half had no other underlying condition. [26] Nivoix and coworkers have also highlighted the role of 'concomitant DM' as a risk factor for IA.[29]

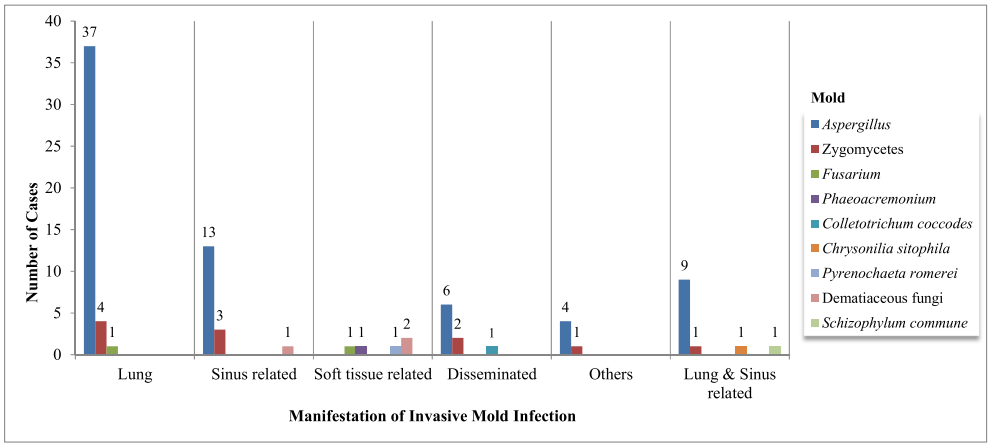

Figure 1. Distribution of Mould by Manifestation. Note that soft tissue infections were caused by a variety of Aspergillus moulds.

Table 8. Treatment/management.

Prophylaxis/empiric treatment	*n* (%)[a]
Prophylaxis	65 (41.9)
Empiric antifungal used	94 (60.6)
Empiric antifungals and antibacterials	80 (51.6)
First empiric antifungal	
Amphotericin B deoxycholate (conventional amphotericin)	48 (31.0)
Lipid preparation of amphotericin	16 (10.3)
Fluconazole	13 (8.4)
Extended-spectrum azole[b]	11 (7.1)
Itraconazole	3 (1.9)
Echinocandin	3 (1.9)
Treatment	***n*** **(%)**
Surgical procedure (other than biopsy)	48 (31.0)
Includes percutaneous drainage procedure	8 (5.2)
Only consisted of antifungals	98 (63.2)
First antifungal for targeted therapy	
Voriconazole	77 (49.7)
Amphotericin B deoxycholate (conventional amphotericin)	22 (14.2)
Lipid preparation of amphotericin	20 (12.9)
Itraconazole	12 (7.7)
Posaconazole	8 (5.2)
Echinocandin	6 (3.9)
Fluconazole	3 (1.9)
Combination therapy	14 (9.0)
Use of iron chelator	1 (0.6)
Use of immunomodulator	4 (2.6)
Treatment duration in weeks, median (range)	8 (0-104)

[a]Expressed as percentage of the total number of subjects ($n = 155$).
[b]Posaconazole or voriconazole.

In addition, DM was also common in patients with more 'classical' underlying conditions, such as hematologic malignancies. This supports the possibility that the presence of two or more risk factors/predisposing conditions may precipitate the occurrence of IMI in persons who do not meet current consensus criteria.[27] In the study, the most common EORTC host factors were prolonged use of corticosteroids and a recent history of neutropenia. These were observed less frequently among patients with DM but more often in those with fever or lung involvement. Indeed, > 90% of neutropenic IMI patients had pulmonary involvement; by contrast, among 33 IMI subjects with only extrapulmonary disease, 87.9% were non-neutropenic. More important, non-neutropenic patients also tended not to have fever. Hence, clinicians should consider IMI in patients with the appropriate presentation, even if they are afebrile and do not have classical risk factors.

In our series, 11.6% of patients had an underlying rheumatologic condition. As these individuals are typically treated with immunosuppressive agents, they may have an elevated risk of opportunistic infections, including IMIs. A recent study suggested that rising use of anti–tumor necrosis factor agents may increase the risk of IMI in patients with rheumatologic conditions, [30] although only 3.9% of patients had received prior monoclonal antibody therapy in the present work. Previous studies have typically not found high IMI rates among patients with rheumatologic conditions. For example, in an analysis of 5,604 patients with juvenile systemic lupus erythematosus (SLE), only 2.1% were diagnosed with IA.[31] Kim et al. found a similarly low incidence of IMIs in a review of 3,159 individuals with SLE or rheumatoid arthritis.[32] Our study shows that patients with rheumatologic conditions may constitute a significant proportion of patients with IMI.

Almost 80% of IMI cases had lung involvement, and more than 60% were only in the lung. Patients with IMI involving the lungs had a wide range of CT features, which aligns with findings from a Korean study.[33] Nucci and colleagues have proposed a new category of aspergillosis called

Table 9. Association between recent history of neutropenia and/or prolonged use of steroids, and other patient variables.

Variable[a]	Recent history of neutropenia			Prolonged use of steroids			Recent history of neutropenia or prolonged use of steroids		
	Yes $n = 60$	No $n = 95$	*P*-value	Yes $n = 61$	No $n = 94$	*P*-value	Yes $n = 99$	No[b] $n = 56$	*P*-value
Age	44.3 ± 20.3	54.5 ± 16.4	**.001**	52.7 ± 16.9	49.1 ± 19.6	.242	49.0 ± 19.1	53.2 ± 17.7	.177
Diabetes	10 (16.7)	38 (40.0)	**.002**	16 (26.2)	32 (34.0)	.304	23 (23.2)	25 (44.6)	**.006**
Fever	53 (88.3)	54 (56.8)	**<.001**	44 (72.1)	63 (67.0)	.501	79 (79.8)	28 (50.0)	**<.001**
Fever persistent despite antibiotics	44 (73.3)	37 (38.9)	**<.001**	34 (55.7)	47 (50.0)	.485	64 (64.6)	17 (30.4)	**<.001**
Any lung involvement	56 (93.3)	66 (69.5)	**<.001**	54 (88.5)	68 (72.3)	**.016**	90 (90.9)	32 (57.1)	**<.001**
Skin/subcutaneous	2 (3.3)	6 (6.3)	.486	3 (4.9)	5 (5.3)	1.000	3 (3.0)	5 (8.9)	.138
Disseminated	6 (10.0)	9 (9.5)	.914	7 (11.5)	8 (8.5)	.542	10 (10.1)	5 (8.9)	.813
Surgery	17 (28.3)	31 (32.6)	.573	10 (16.4)	38 (40.4)	**.002**	22 (22.2)	26 (46.4)	**.002**
Survival (alive at discharge)	36 (60.0)	65 (70.7)	.174	33 (55.0)	68 (73.9)	**.016**	58 (59.2)	43 (79.6)	**.011**

P values are based on two-sample t-test for continuous variables and χ^2/Fisher exact test for categorical variables.

[a]Data shown are mean ± standard deviation or frequency (proportion).

[b]Includes only patients who did not have recent history of neutropenia *and* did not have prolonged use of steroids.

'IA without prespecified radiologic criteria' and shown that these patients have comparable clinical characteristics and outcomes to those with EORTC/MSG proven and probable IA.[16] Girmenia et al. have reported similar experiences.[17] The present work adds further weight to the suggestion that IMI should not be ruled out in patients with a suspected infection whose chest features on CT do not fit the EORTC/MSG criteria.

As expected, *Aspergillus* spp. were responsible for the majority of IMIs. *A. fumigatus* was the most commonly isolated species, although *A. flavus* was as common in India. This has been noted previously[7] and may relate to the hot climate.[34] Indeed, an aerobiological survey in Delhi found that *A. flavus* was the most common *Aspergillus*.[35] *A. flavus* was also the predominant *Aspergillus* in a Sudanese study.[36] The predominance of *A. flavus* in sinusitis has been attributed to its large conidia,[34] which may affect pathogenesis. However, the role of climate is less straightforward, given that Singapore and Thailand are hot year-round and yet *A. fumigatus* remains the leading mould, as in temperate areas. Interestingly, at 20 and 30°C, the germination rates of various species of *Aspergillus* have been found to be similar, but at 41°C the germination rate of *A. fumigatus* was enhanced, whereas that of *A. flavus* fell by 45%.[37] The Delhi survey noted that mould concentrations in the air were lowest in the dry and hot summer months and rose at times of precipitation.[33] The authors suggested that high humidity was a factor, and it could also contribute to the *Aspergillus* isolation pattern in the present work.

The most commonly used antifungal for empirical therapy was AMB, possibly owing to its lower cost. However, there were large differences between locations, with no use of AMB for empiric therapy recorded in the Chinese and Singaporean centers, largely due to nephrotoxicity concerns. The frequency of empiric therapy was also variable, possibly due to heightened suspicion at some centers (e.g. Thailand, India), unexpected culture results (e.g. peritoneal dialysis fluid in Singapore), and a small contribution from AML and HSCT cases in other centers (Singapore, China).

The 90-day mortality rate (32.9%) is an improvement on historical data. For example, a review of case series published between 1995 and 1999 placed the overall case-fatality rate for IA at 58%;[38] it was even higher in HSCT recipients (86.7%) and in patients with disseminated disease (88.1%). However, mortality rates appear to be declining. The TRANSNET report, covering the years 2001–2006, noted a 1-year survival of 59% in solid organ transplant recipients with IA and of 61% in patients with non-*Aspergillus* moulds.[39] An Italian report, limited to IA in patients with acute leukemia, demonstrated a falling mortality rate, from 48% in a 1987–1998 cohort, to 38.5% in a 1999–2003 cohort, to 13% in those studied post-2006.[5] In our series, the 90-day survival of aspergillosis cases among acute leukemics was 59.5%.

The Italian group attributed the improving survival rates to prompt diagnostics and possibly antifungal prophylaxis.[5] The authors of an Austrian study attributed improved survival of IA patients to increased use of VCZ.[6] Several factors may contribute to the survival rates in the present series: all participating sites were tertiary centers with experience in managing infections in immunocompromised hosts; the adoption of newer diagnostic techniques, such as

the GM assay; and the use of newer agents, such as VCZ. The presence of a substantial number of patients without recent neutropenia or prolonged steroid use as risk factors ($n = 56/155$) may also have contributed, as these individuals were more likely to survive to discharge. These 'non-classical' hosts often had unusual conditions (e.g., peritonitis or soft tissue infection), in which a specific intervention (e.g., removal of a Tenchkoff catheter) likely contributed to a positive outcome.

The study had several limitations. Most importantly, it was retrospective in design. However, given scarce manpower and the need to include subjects from many sources (positive GM, culture, histopathology), retrospective review of microbiology, and histopathology logs was an effective means of ensuring consecutive cases were recruited. The study was also relatively short in duration, spanning 1 year (2012). Hence, its findings may reflect the peculiarities of the different centers in that period. For example in Taiwan, typhoons are known to increase fungal colony counts in the air, and thus local weather patterns may have affected the results.[40] However, 2012 was considered an average year for typhoons.[41]

Despite these limitations, this study provides a valuable insight into IMIs from five centers in five different Asian countries, a new milestone for the region. Looking ahead, as electronic medical records in hospitals become more sophisticated, this may facilitate common standards in data archives and data collection across hospital centers. Subsequently, conducting multicenter studies may be made more efficient along with augmented data quality. This highlights the value of pioneering cross-country collaboration research—providing a basis for enhanced collaboration in future, aided by technology. In conclusion, IMI appears to a significant clinical problem and occurs in a wide variety of hosts. Invasive aspergillosis was the most common infection. DM (particularly in patients without a history of steroid exposure or recent neutropenia) and rheumatologic diseases were frequently observed underlying conditions, highlighting the importance of appropriate surveillance in these individuals. Unsurprisingly, there were important differences between centers in the types of moulds isolated, the disease spectrum, and the clinical management of suspected and confirmed cases.

Supplementary material

Supplementary data are available at *MMY* online.

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