Invasive Fungal Infection in Ramathibodi Hospital: A Ten-Year Autopsy Review

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Objective: *Determine the clinicopathological findings in autopsy cases with invasive fungal infection.*
Material and Method: *The autopsy and medical records with invasive fungal infection in Ramathibodi Hospital between January 1997 and December 2006 were analyzed. The criterions for the diagnosis of invasive fungal infection were the evidence of fungal elements from histopathological section. The age, gender, underlying predisposing risk factors for the disease, clinical manifestations, extent of systemic organ involvement documented morphologically at autopsy, and fungal culture were analyzed.*
Results: *There were 155 autopsy cases (73 male, 82 female; mean age 45.3 years, range 3 months to 87 years) with the diagnosis of invasive fungal infection. The common clinical presentations were fever (55.5%), and dyspnea (26.5%). The invasive fungal infection was associated with hematologic malignancy in 31%. The common mycoses were aspergillosis and candidiasis, which were observed in 88 and 80 cases, respectively. There were 32 cases (20.6%) of mixed fungal infection. Cultures from autopsy materials were positive for fungus in 80 cases out of 99 cases (80.8%). The most frequent site of fungal infection was in the lungs (74.8%), followed by gastrointestinal tract (28.4%), and brain (26.5%). Invasive fungal infection was diagnosed intravitally in 63.9% of total cases.*
Conclusion: *A diagnosis of invasive fungal infection requires a high index of suspicion, especially in immunocompromised patients who presented with prolonged fever. Clinical specimens must be sent for histopathology and fungal culture for a definite diagnosis and an appropriate management. Therefore, the physician should inform the laboratory if invasive fungal infection is suspected because special media are necessary for the best recovery of fungi. In addition, the present study underscores the significance of autopsy as a diagnostic method and means of medical quality control.*

Keywords: *Invasive fungal infection, Mycoses, Aspergillosis, Candidiasis, Autopsy*

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Invasive fungal infection (IFI) has emerged as a major cause of morbidity and mortality in the hospital. IFI is difficult to diagnose and it encompasses a wide clinical spectrum of disease[1]. The definite diagnosis largely relies on bioptic procedures with histological and cultural confirmation of fungal organism from infected tissues[1-3]. Early recognition of this potentially fatal disease, therefore, takes on a greater urgency than before, particularly when effective treatment is now available. Early antifungal therapy improves the outcome of the disease and reduces the associated mortality and morbidity. At present, there are only a few epidemiologic data regarding the fungal agents causing IFI in Thailand[4,5]. In the present paper, the

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authors present 155 cases of histologically verified IFI seen in Ramathibodi Hospital over a decade. The autopsy rate among all hospital deaths at Ramathibodi Hospital between 1997 and 2006 was 15 to 20%.

Material and Method

This was a retrospective of IFI diagnosed on academic autopsy material from the Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, during January 1997 to December 2006. All academic autopsy cases were seronegative for human immunodeficiency virus (HIV). Medicolegal autopsies were not included in the present study. During the ten-year period of the study, 1,652 autopsies were obtained, most of which were adults.

All tissues were formalin-fixed and routinely processed for paraffin-embedding. Tissue sections, 4 um thick, were cut. Routine hematoxylin and eosin (H&E)-stained sections were examined for histopathologic findings. Fungal morphology was delineated using special stains i.e. Gomori's methenamine silver (GMS), and periodic acid Schiff (PAS). The histopathological diagnosis of IFI was reviewed. Information obtained from the autopsy and medical records including age, gender, underlying predisposing risk factors for the disease, clinical manifestations, extent of systemic organ involvement documented morphologically at autopsy, and any fungal species that had been isolated from the patients, either premortem or postmortem were analyzed using descriptive statistics: percentage, mean, median and standard deviation to present the results. The present study was approved by the committee on human research at Ramathibodi Hospital (ID06-49-10).

Results

Patient characteristics

There were 1,652 medical autopsy records in Ramathibodi Hospital during the years 1997-2006. IFI was diagnosed by the presence of invasive fungal organisms in the tissues by H&E and special stains (GMS and PAS) in 155 cases. There were 73 males and 82 females (Table 1). The ages of the patients ranged from 3 months to 87 years with the mean and median ages of 45.3 and 48 years, respectively.

Clinical characteristics

The onset of symptoms ranged from twelve hours to four months, 56.8% of cases were shorter than one week and 17.4% were longer than one month. Therefore, IFI can present as an acute, subacute and chronic infection. The body temperatures (at the admission time) varied from 35□C to 40.5□C. Fifty percent had a body temperature higher than 38□C. The mean and median body temperatures (at admission) were 37.87□C and 37.9□C, respectively. The details of the patients' characteristic are presented in Table 1. The chief symptoms were fever (86 cases, 55.5%), dyspnea (41 cases, 26.5%), cough with or without mucopurulent sputum (10 cases, 6.5%), diarrhea (8 cases, 5.2%), alteration of consciousness (6 cases, 3.9%), fatigue (6 cases, 3.9%), jaundice (6 cases, 3.9%), headache (5 cases, 3.2%), nausea/vomiting (5 cases, 3.2%), abdominal pain (4 cases, 2.6%), blurred vision (4 cases, 2.6%), or chest pain (2 cases, 1.3%), are listed in Table 2. The underlying diseases and/or co-infection are shown in Table 3. Hematologic malignancy (48 cases, 31%) including myeloproliferative and lymphoproliferative

Table 1. Patients' characteristics with invasive fungal infection (n = 155)

Detail	No. of patients	Percent
Age, years, mean ± SD	45.3 ± 22.7	
Gender		
Male	73	47.1
Female	82	52.9
Duration of chief complaint		
≤ 1 week	88	56.8
1 week to 1 month	40	25.8
≥ 1 month	27	17.4
Temperature at admission, □C, mean ± SD (n = 88)	37.87 ± 1.17	
≥ 38□C	44	50.0
Neutropenia (n = 129)	63	48.8
Corticosteroids treatment	31	20.0
History of organ transplant	7	4.5

Table 2. Chief symptoms of the autopsy cases with invasive fungal infection (n = 155)

Chief symptoms	No. of patients*	Percent
Fever	86	55.5
Dyspnea	41	26.5
Cough with or without mucopurulent sputum	10	6.5
Diarrhea	8	5.2
Alteration of consciousness	6	3.9
Fatigue	6	3.9
Jaundice	6	3.9
Headache	5	3.2
Nausea/vomiting	5	3.2
Abdominal pain	4	2.6
Blur vision	4	2.6
Chest pain	2	1.3

* More than one symptoms in a case

Table 3. Underlying diseases and/or co-infection of the autopsy cases with invasive fungal infection* (n = 155)

Underlying diseases	No. of patients	Percent
Myeloproliferative disorders	24	15.5
Lymphoproliferative disorders	24	15.5
Other hematologic diseases**	12	7.7
SLE	23	14.8
Diabetes mellitus	18	11.6
Hypertension	10	6.5
Cirrhosis	8	5.2
Solid malignancy	7	4.5
Tuberculosis	4	2.6
Cytomegaloviral infection	3	1.9
Chronic renal failure	3	1.9
Wilson disease	2	1.3
Malnutrition	1	0.6
Myasthenia gravis	1	0.6
Severe combine immunodeficiency disease	1	0.6

* The patients may have one or more than one underlying diseases

** Including thalassemia (documenting by hemoglobin typing) 5 cases, reactive hemophagocytic syndrome 3 cases, aplastic anemia 2 cases, idiopathic thrombocytopenic purpura with splenectomy 2 cases

disorders were common. The other hematologic diseases including thalassemia (documented by hemoglobin typing) five cases, reactive hemophagocytic syndrome three cases, aplastic anemia two cases, and idiopathic thrombocytopenic purpura with splenectomy two cases were identified.

In the present series, neutropenia (neutrophils in peripheral blood < 500 cells/mm^3) was described in 49% of cases. Another factor in the development of IFI was immunosuppression due to corticosteroids administered (20%). Seven cases had a history of organ transplantation and placed on immunosuppressive agents. Associated tuberculosis and opportunistic cytomegalovirus (CMV) infection was represented in four and three cases, respectively. In most cases, death was related to extensive pulmonary involvement or fungal dissemination. The antemortem diagnosis by clinicians was 63.9% corrected regarding retrospective analysis of autopsy examination.

Organ involvements

The extent of systemic organ involvement

documented morphologically at autopsy included lungs (116 cases, 74.8%), brain (41 cases, 26.5%), heart (29 cases, 18.7%), stomach (28 cases, 18.1%), trachea (22 cases, 14.2%), thyroid gland (19 cases, 12.3%), liver (17 cases, 11%), large intestine (17 cases, 11%), esophagus (16 cases, 10.3%), and others, as shown in Table 4.

Histopathology

The histopathologies documenting invasive fungal infections were as follow Asper gillosis (88 cases), Candidiasis (80 cases), Cryptococcosis (5 cases), Histoplasmosis (1 case), Mucormycosis (8 cases), Phaeohyphomycosis (1 case), Pneumocystosis (5 cases), and Trichosporonosis (1 case) (Table 5).

Microbiology

Cultures from autopsy materials that determined the presence of fungus were positive in 80 cases out of 99 cases (80.8%) Additionally, the five common fungi isolated were *Candida* spp. (51 cases), *Aspergillus* spp. (34 cases), *Mucor* spp. (3 cases), *Cryptococcus neoformans* (1 case), and *Trichosporon cutaneum* (1 case). The predominant IFI demonstrating by histopathology is A*spergillus,* whereas *Candida* was the most common IFI demonstrating by culture (Table 5). Multiple *Candida* spp. were implicated as causative agents including *Candida albicans* (35 cases), *Candida tropicalis* (14 cases), *Candida glabrata* (3 cases), *Candida parapsilosis* (2 cases), *Candida guilliermondii*

Table 4. Infected organs with invasive fungal infection, Aspergillosis and Candidiasis, as evidenced by histopathology

Infected organs	IFI (n = 155)		Aspergillosis (n = 88)		Candidiasis (n = 80)	
	No. of patients	Percent	No. of patients	Percent	No. of patients	Percent
Lung	116	74.8	81	92.0	54	67.5
Brain	41	26.5	30	34.1	18	22.5
Heart	29	18.7	16	18.2	18	22.5
Pituitary gland	2	1.3	2	2.3	0	0
Sinonasal area	5	3.2	4	4.5	0	0
Optic nerve	1	0.6	1	1.1	0	0
Eye	1	0.6	1	1.1	0	0
Thyroid gland	19	12.3	14	15.9	9	11.3
Pharynx	2	1.3	1	1.1	2	2.5
Epiglottis	2	1.3	1	1.1	2	2.5
Trachea	22	14.2	9	10.2	16	20.0
Pleura	6	3.9	5	5.7	1	1.3
Tongue	10	6.5	2	2.3	9	11.3
Submandibular gland	1	0.6	0	0	1	1.3
Palatine tonsil	1	0.6	0	0	1	1.3
Lymph node	10	6.5	9	10.2	4	5.0
Kidney	14	9.0	6	6.8	8	10.0
Urinary bladder	5	3.2	3	3.4	4	5.0
Liver	17	11.0	12	13.6	11	13.8
Spleen	11	7.1	8	9.1	9	11.3
Gallbladder	1	0.6	1	1.1	0	0
Pancreas	6	3.9	6	6.8	2	2.5
Adrenal gland	6	3.9	5	5.7	1	1.3
Esophagus	16	10.3	5	5.7	14	17.5
Stomach	28	18.1	12	13.6	21	26.3
Small intestine	14	9.0	6	6.8	11	13.8
Large intestine	17	11.0	8	9.1	12	15.0
Vermiform appendix	1	0.6	1	1.1	0	0
Aorta and its branches	3	1.9	2	2.3	0	0
Diaphragm	10	6.5	8	9.1	5	6.3
Psoas muscle	4	2.6	4	4.5	1	1.3

IFI = invasive fungal infection

Table 5. Invasive fungal agents as evidenced by histopathology and culture (n = 155)

Diagnosis by hispathology	No. of patients	Culture		
		Positive	Negative	Not available
Single infection				
Aspergillosis	60	24	12	24
Candidiasis	52	33	3	16
Cryptococcosis	2	0	1	1
Histoplasmosis	1	0	0	1
Mucormycosis	4	1	1	2
Pneumocystosis	2	-	-	2
Pheohyphomycosis	1	0	1	0
Trichosporonosis	1	1	0	0
Mixed infection				
Aspergillosis + Candidiasis	23	16*	0	7
Aspergillosis + Mucormycosis	2	1**	0	1
Aspergillosis + Pneumocystosis	1	0	1	0
Candidiasis + Cryptococcosis	1	1***	0	0
Candidiasis + Mucormycosis	1	0	0	1
Candidiasis + Pneumocystosis	1	1***	0	0
Cryptococcosis + Pneumocystosis	1	0	0	1
Aspergillosis + Candidiasis + Cryptococcosis	1	1****	0	0
Aspergillosis + Candidiasis + Mucormycosis	1	1*****	0	0
Total cases	155	80	19	56

* *Aspergillus* spp.: 1 case, *Candida* spp.: 7 cases, *Aspergillus* spp.: + *Candida* spp. 8 cases;
** *Mucor* spp.: 1 case; *** *Candida* spp.: 1 case; **** *Candida* spp. + *Cryptococcus* spp.: 1 case;
***** *Aspergillus* spp. + *Candida* spp. + *Mucor* spp.: 1 case

(1 case), *Candida krusei* (1 case), and other species of *Candida* (2 cases). Multiple *Aspergillus* spp. including *Aspergillus fumigatus* (21 cases), *Aspergillus flavus* (8 cases), *Aspergillus niger* (1 case), and other species of *Aspergillus* (5 cases) were also implicated as causative agents.

Discussion

IFI is a common opportunistic infection in immunosuppressed and debilitated hosts that can cause serious or disseminated disease. The incidence of IFI among autopsy cases is widely varied, ranging from 1 to 30.9%, however, small numbers and various study groups have biased many of these estimates[4-10]. Most of the reported cases of IFI were associated with pre-existing immune compromise ranging from alcoholism and diabetes to organ transplantation[4-13].

Most of the previously reported patients showed neutropenia (23.4-63.6%)[8,10], and received corticosteroids (13-63.6%)[4,5,10]. The combination of degree and duration of neutropenia is the most important predisposing host factor. In the present study, the most common presentation was fever (≥ 38□C), followed by dyspnea, cough, diarrhea, alteration of consciousness, fatigue, jaundice, headache, nausea/vomiting, abdominal pain, and blur vision. The duration of these presenting symptoms was highly variable ranging from days to months. Most patients had respiratory symptoms, clinical signs, laboratory and radiological features resembling bacterial pneumonias. Hence, the diagnosis of IFI cannot be made clinically in any of these patients. A high index of suspicion is, therefore, very essential, especially in immune depleted cases.

An important finding from the epidemiologically analyzed data is that hematologic malignancy is the major disease underlying IFI (13.4-38.5%)[7,9,10]. The most important factor that predisposed malignant hematologic patients to infection is the marked reduction in the number of normal circulating phagocytic cells and neutrophils. Long periods of profound neutrophils depletion have been identified as an important

factor predisposing malignant hematologic patients to fungal infections such as aspergillosis and candidiasis. Treatment with corticosteroids has been identified as an important factor to fungal infection[13]. The authors' findings were similar to previous observation regarding the association between IFI and neoplastic diseases and corticosteroids therapy.

The numbers of various forms of IFI have increased markedly over the past decade. Reviews of previous reported cases indicated a preferential pulmonary involvement of 14.3-68.1%[6,7,9]. The authors' report had a high incidence of pulmonary involvement (74.8%). The most common pulmonary manifestation was pneumonia, followed by lung abscesses. Fungi can disseminate to virtually any organ, including gastrointestinal tract, brain, heart, and others (50-52%)[6, 7].

The authors observed a difference in organ distribution among *Aspergillus*. The pulmonary and bronchial systems were involved most frequently. This suggests that the lung and bronchi are at the highest risk of being exposed to *Aspergillus*. *Aspergillus* has a predilection to invade blood vessel walls and produce vasculitis and mycotic aneurysm. In the present study, angio-invasive *Aspergillus* was commonly found in the lungs, which cause pulmonary hemorrhage. The angio-invasive nature of *Aspergillus* is directly related to its ability to digest elastic tissue, a characteristic mediated by production of elastase enzymes[14,15]. The vascular walls that contain elastic tissue are destroyed and the hyphal growth in vascular wall compromises the structural integrity, resulting in aneurysm formation, pulmonary hemorrhage[14,15]. The growth of fungal hyphae can produce thromboembolism, resulting in infarction. The diagnosis cannot be made by clinical manifestations, but can only be judged by histopathologic demonstration of fungal elements and tissue reactions in a biopsy. The obtained tissue may show only necrotic debris from infarcted areas without the characteristic of aspergillus hyphae. Therefore, the diagnosis may be strongly apprehended only by a judicious interpretation of the clinical and radiological findings in the presence of the most important risk factors for invasive aspergillosis.

Central nervous system fungal infection occurred in 4.3-17.8% of cases[6,9] and is more common after hematogenous dissemination from the lungs than direct spread from the sinonasal area. The most common fungal pathogen in a previous report was *Aspergillus*[16]. The authors' report confirmed previous findings. In addition, there were four cases of sinonasal aspergillosis directly invading the skull base into the brain parenchyma. *Aspergillus fumigatus* were the most frequent causative agents in both phenomenons.

In the present study, the predominant IFI demonstrated by histopathology was *Aspergillus*, whereas *Candida* was the most common IFI demonstrated by culture. The results can be clarified since using basic bacterial culture media such as chocolate agar, eosin-methylene blue agar or blood agar, either alone or in combination, would have reduced detection of filamentous fungi such as *Aspergillus fumigatus*, while pathogenic *Candida* spp. can grow rather easily on these basic media[17]. Sabouraud dextrose agar is the most appropriate medium for fungal culture[18]. These findings reveal that *Aspergillus* can be detected easier by histopathology than culture. Therefore, the physician should inform the laboratory whenever IFI is suspected since fungal culture media are necessary for the best recovery of filamentous fungi.

IFIs were clinically diagnosed in 63.9% of cases. In previous reports, infection was found to be the most frequently overlooked diagnosis, accounting for 31-45% of misdiagnosed cases[6,19-22]. The increase use of immunosuppressive therapy has been complicated by serious IFIs. Their unusual clinical manifestations and the masking of signs and symptoms by another serious condition also resulted in these fungal infections being missed in such cases.

IFI is a serious infection with high morbidity and mortality rate. Early diagnosis and prompt treatment are essential. It has a wide spectrum of clinical manifestations. Current advances in medicine result in an increasing population of severe immunocompromised patients at high risk for IFI. However, recent technology has not improved the overall accuracy of clinical diagnosis of IFI. Therefore, histopathology and culture of autopsy specimens may reveal major unexpected findings that are of clinical importance and emphasis on autopsy evaluation in that it is necessary for the improvement of diagnosis, treatment and prevention of infectious diseases. The present study may provide useful information concerning infectious diseases among patients undergoing autopsy. Fungal agents can disseminate virtually to any parts of the body, and indeed the true extents of these spreads are often only apparent at autopsy. The present study offers the unique opportunity to assess the epidemiology of IFI over a ten-year period in Ramathibodi Hospital.

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การศึกษาการติดเชื้อราในร่างกายชนิดลุกลามจากการตรวจชันสูตรศพทางพยาธิวิทยา 10 ปี ย้อนหลังที่โรงพยาบาลรามาธิบดี

นพดล ลาภเจริญทรัพย์, สหภูมิ ศรีสุมะ, ธนัช งานประเสริฐศรี, รังสิมา อรุณโรจน์, พิริยาภรณ์ จงตระกูล, พิทักษ์ สันตนิรันดร์, ธำรง จิรจริยาเวช, วรชัย ศิริกุลชยานนท์

วัตถุประสงค์*: ศึกษาลักษณะทางพยาธิวิทยาและทางคลินิกของการติดเชื้อราในร่างกายชนิดลุกลาม จากการตรวจชันสูตรศพทางพยาธิวิทยา*

วัสดุและวิธีการ*: ศึกษารายงานการตรวจชันสูตรศพทางพยาธิวิทยาและเวชระเบียนผู้ป่วยเสียชีวิตในโรงพยาบาลรามาธิบดี ตั้งแต่ ปี พ.ศ. 2540 ถึง พ.ศ. 2549 ซึ่งได้ทำการตรวจศพโดยพยาธิแพทย์ และได้รับการวินิจฉัยเป็นการติดเชื้อราในร่างกายชนิดลุกลาม มีเกณฑ์การวินิจฉัยโรคคือ การพบเชื้อราลุกลามเข้าไปในเนื้อเยื่อโดยการตรวจทางจุลพยาธิวิทยา รายงานนี้ทำการศึกษาข้อมูลด้านอายุ เพศ โรคประจำตัว อาการแสดง การแพร่กระจายของเชื้อราในอวัยวะภายใน และผลการเพาะเชื้อ*

ผลการศึกษา*: ผลการตรวจชันสูตรศพทางพยาธิวิทยา 155 ราย (เพศชาย 73 ราย เพศหญิง 82 ราย อายุเฉลี่ย 45.3 ปี ช่วงอายุตั้งแต่ 3 เดือน ถึง 87 ปี) ที่วินิจฉัยเป็นการติดเชื้อราในร่างกายชนิดลุกลาม พบว่าอาการที่ผู้ป่วยมาพบแพทย์ สองอันดับแรก คือ ไข้ (55.5%) และ หอบเหนื่อย (26.5%) ผู้ป่วยมีโรคประจำตัวที่พบบ่อยมากที่สุด คือ มะเร็งระบบเลือด โดยพบประมาณ ร้อยละ 31 เชื้อราที่พบมาก คือ Aspergillus spp. (88 ราย) และ Candida spp. (80 ราย) พบว่า 32 ราย ร้อยละ 20.6 มีการติดเชื้อราตั้งแต่สองชนิดขึ้นไป การเพาะเชื้อราจากสิ่งส่งตรวจแล้วพบเชื้อรา 80 รายจากผู้ป่วย 99 ราย คิดเป็นร้อยละ 80.8 อวัยวะภายในที่ติดเชื้อราในร่างกายชนิดลุกลาม ที่พบบ่อยได้แก่ ปอด (74.8%) ทางเดินอาหาร (28.4%) สมอง (26.5%) และอวัยวะอื่น ๆ โดยการติดเชื้อรา สามารถวินิจฉัยในขณะผู้ป่วยมีชีวิตอยู่ร้อยละ 63.9*

สรุป*: การติดเชื้อราในร่างกายชนิดลุกลามพึงสงสัยในผู้ป่วยที่มีภูมิคุ้มกันต่ำมีไข้มานานแพทย์จึงควรส่งตรวจชิ้นเนื้อและการเพาะเชื้อราเพื่อให้ได้รับการวินิจฉัยและการรักษาที่เหมาะสม โดยแจ้งแก่ห้องปฏิบัติการ เพื่อห้องปฏิบัติการจะได้เลือกอาหารเลี้ยงเชื้อราที่เหมาะสม นอกจากนี้รายงานนี้ยังประเมินคุณค่าของการตรวจชันสูตรศพทางพยาธิวิทยาในการเป็นเครื่องช่วยวินิจฉัยโรคตลอดจนควบคุมคุณภาพการตรวจวินิจฉัย*