UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
Faculdade de Farmácia
Disciplina de Trabalho de Conclusão de Curso de Farmácia

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Amanda Zamboni

Porto Alegre, junho de 2018.

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"A mente despreparada não consegue ver a mão estendida da oportunidade."

(Alexander Fleming)

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CASES OF MYCOSES IN IMMUNOCOMPETENT PEOPLE CONFIRMED THROUGH AUTOPSY: A NARRATIVE REVIEW OF 10 YEARS

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ABSTRACT

Fungi are microorganisms that cause primarily non-life threatening infections in immunocompetent hosts and invasive and fatal ones in immunocompromised subjects. Cancer patients, autoimmune diseases carriers, transplant recipients, HIV/AIDS patients or people under treatment with immunosuppressive agents are at higher risk of developing invasive mycoses. Although not common, immunocompetent people can develop fungal infections with fatal outcomes. The purpose of this review is to verify the occurrence of death by mycoses in immunocompetent humans in cases confirmed through autopsies published within the last ten years.

KEYWORDS

Mycosis, autopsy, immunocompetent

INTRODUCTION

Fungi are eukaryotic organisms that can exist in the form of independent single cells, yeasts,and can be based on hyphal threads,molds[1]. They can infect humans and cause various diseases from superficial infections of the skin and mucosal surfaces to invasive infections of internal organs [2]. In immunocompetent subjects, these microorganisms cause primarily superficial or non-life threatening infections [3], since the immune response is activated in order to eliminate the pathogen [4], while more invasive and fatal in immunocompromised people [5]. Amongst this group, transplant recipients have a significant risk of invasive fungal diseases [5], since they are under treatment with immunosuppressive drugs [6]. Other groups at risk include people with HIV [7], haematological malignancies [8, 9], patients with autoimmune diseases [10] and any other under treatment with corticosteroid agents [11, 12]. Chronically ill patients, like those with liver diseases and diabetes are also at risk of developing mycoses [13].

Amongst the causes of mycoses in all populations, major fungal pathogens include *Candida albicans, Cryptococcus neoformans, Aspergillus fumigatus* and *Histoplasma capsulatum*[2], and a simple laboratory tool to detect these fungal infections is to test for 1-3 β D glucan[14], which is a major constituent of fungal cell walls[15], in patient's samples. Many cases of fungal infections are mistaken with bacterial, so Gram Stain, although non-specific, is important to rule out any bacterial diagnosis before initiating antimicrobial treatment [16].

When in life diagnosis is not possible, it is important to have autopsies performed to rate the incidence of these infections [1].These results are of fundamental importance to define healthcare policies [17] and also correlate them to the clinical signs and symptoms the patients present upon hospital admission so that clinicians can direct the course of treatment in a more successful manner to save the new patient's lives.

The purpose of this review is to verify the occurrence of death by mycoses in immunocompetent humans in cases confirmed through autopsies published within the last ten years.

MATERIALS AND METHODS

This is a narrative review of literature.

Article search

All articles used in this review were searched on Pubmed using the following keywords: *mycoses* AND *autopsy*. A 10 years filter was included.

Inclusion criteria

All articles included in this review were case reports of immunocompetent humans with death and autopsy findings of mycoses.

Exclusion criteria

Immunocompromised people, including diabetics, transplant recipients, solid cancer and blood malignancies patients, HIV/AIDS, autoimmune disease subjects, people under treatment with corticosteroids, people with cirrhosis or history of tobacco abuse, were excluded from this review.

RESULTS

The research using the described terms generated 227 results of which only 10 were eligible for this review, comprising 10 cases reports of death by mycoses confirmed through autopsy. The summarized compilation of the cases that matched our inclusion criteria is presented in table 1.

Table 1. Fatal cases of mycoses confirmed with autopsy

Year	**Author**	**Subject** Age, Gender	**Symptoms/complaints upon hospital admissions**	**Diagnosis**	**Fungi species**
2010	Ortmann et al	53, F	Hypothermia and asystolia	C	*Pseudallescheria boydii*
2010	Terada	36, M	Hemoptysis and mental sickness	C	*Cryptococcus neoformans*
2012	Kawakami	68, F	Gradual mental deterioration, dyspnea and cardiac arrest one day after admission	L	*Aspergillus fumigatus*
2012	Ochiai et al	21, F	Mild neck stiffness without neurological symptoms	A	Phaeohyphomycosis*
2013	Ubesie et al	13, F	Neck masses of 3 years, body swelling of 3 months and reduction in urinary output of 2 months	A	*Histoplasma capsulatum*
2014	Chatterjee	29, M	Low grade intermittent fever of 3 months with occasional rigor	A	*Aspergillus flavus*
2014	Ramsi et al	13, M	Influenza-like symptoms, fever, abdominal and neck pain. Febrile, tachycardic, hypotensive and respiratory distress.	A	*Coccidioides* spp.
2015	Parikh et al	70, M	Abdominal pain and inability to pass stool for 8 days	A	*Aspergillus* spp.

2015	Yuan and Prayson	55, F	Headaches behind left eye, blurred vision and scotoma	A	*Aspergillus* spp.
2017	Bonsignore et al	43, F	Fever of unknown origin	A	*Histoplasma capsulatum*

F= female; M= male; A= autopsy; C= culture; L=laboratory

*type of fungal infection

In 2010, Ortmann and col reported the case of a 53-year-old woman that plunged into a canal in the Netherlands and was submerged for 20 minutes. At resuscitation, there occurred severe aspiration of mud and water, severe hypothermia and asystole until successful resuscitation. For the next 6 weeks, she was treated with respiratory therapy. Bronchopneumonia was successfully treated with antibiotics. *Candida albicans*, *Burkholderia cepacian* and *Pseudallescheria boydii* were cultured following bronchoalveolar lavage. Tomography showed interstitial affection of the lungs and nephrocalcinosis. With antimycotic therapy, she recovered well. Until she was discharged, she complained about neck pain and headaches. She continued the antifungal therapy at home. She started feeling nausea and gastric pain was readmitted in the hospital and presented hyponaltremia and hypocalcemia and therapy was discontinued. She was then discharged but soon later readmitted again with nausea, vomiting and vertigo, presenting hyper fibrinogemia. Abdominal sonography showed an aneurism of the abdominal aorta. Brain tomography showed enhancements surrounding the brain ventricles, a hydrocephalus and, and signs of local pressure and she started having double vision. A new tomography showed stenosis of the middle cerebral artery and accumulation of cerebral liquor, that showed increase of cells and proteins, indicating inflammation. She then suffered from a severe subarachnoid bleeding from an unknown aneurism of the posterior cerebral artery and died 25 days after the last hospitalization. Multiple fungal aneurisms were found affecting the posterior cerebral arteries, the posterior communicating arteries, and the bifurcations of the left internal carotid artery. After death, *P. Boydii* was cultured from heart blood and from aortic tissue [18].

Tadashi Terada, in 2010, described the case of a 36-year-old Japanese man that was a company office worker and lived in a city where *Cryptococcus* is not endemic. His past history included lumbar disk herniation, bullae of lung, and pneumothorax. His family showed no fungal or immunosuppressive diseases. He had no history of travelling to foreign countries or endemic areas. He wasn't obese, diabetic, asthmatic nor user of steroids and other drugs. Chest X-ray revealed a small cavity in the left lower lobe. Brain computed tomography (CT) showed edema, but no mass lesions. He developed fever, somnolence, nuchal stiffness, positive abnormal neurological reactions, such as Babinski and Kernich reactions, and increased muscular tonus. He soon went into a coma and received a respirator. *Cryptococcus neoformans* was detected in liquor on day 11. Leukocytes count was low and serum total protein, albumin and globulin were almost normal. In

blood, he presented low total protein, low total cholesterol and high glucose. He was treated with intravenous amphotericin B, but died on day 18 after hospitalization [19].

Kawakami and col (2012) reported a case of disseminated aspergillosis associated with tsunami lung (a disorder contracted after near drowning) in a 68-year-old woman that almost drowned in Japan after the 2011 earthquake. She was previously healthy and had no medical history. She was admitted into a hospital 7 hours after the near drowning experience with clear consciousness and ability to walk by herself. She gradually developed dyspnea and mental deterioration that was followed by cardiac arrest with resuscitation on the next day after admission. She was then transferred into the authors' hospital where she was intubated and sedated. Blood tests were altered and 1-3 β D glucan levels were not assessed since fungi infections weren't suspected for she wasn't immunocompromised. Imaging exams showed diffuse bilateral infiltrates and posterior atelectasis in the chest. Head tomography showed no abnormalities and cardiovascular monitoring showed elevated levels of extravascular lung water and increased pulmonary vascular permeability index. Despite antibiotics treatment, her respiratory function did not improve, so the presence of 1-3 β D glucan was tested on day 10 and presented elevated. It was started antifungal treatment, but her conditions continued to worsen. She died on day 18 [20].

Ochiai and col reported, in 2012, a case of cerebral phaeohyphomycosis in a 21-year-old house wife with no contributory history that experienced an acute episode of pyrexia and headache. On admission, she was alter and had mild neck stiffness without neurological symptoms. Imaging exams showed no abnormalities and liquor presented altered levels of cell count, protein and glucose and high 1-3 β D glucan level, indicating fungal infection. She was diagnosed with fungal meningitis, administered voriconazole and her condition improved, having her discharged from the hospital. However, she returned two other times to the hospital with signs of meningitis, had antifungal systemically administered and was discharged. She was given a diagnosis of *Aspergillus niger* infection, even though no culture was made. After 43 months after onset of symptoms, she died. On autopsy, it was identified multiple sharply branched septate hyphae with melanin pigments. From these findings, *A. niger* infection was ruled out and phaeohyphomycosis was diagnosed [21].

Ubesie and col reported a case in 2013 of a 13-year-old girl that was admitted for 16 days and presented neck masses of 3 years duration, generalized body swelling of 3 months and had a reduction in urinary output for 2 months. She took native medications for 1 year in the course of

illness and had 2 episodes of convulsion prior to hospitalization. There was no history of using antibiotic drugs, chemotherapy or steroids that may have caused immunosuppression. Tests for HIV and tuberculosis were negative. She had respiratory distress, looked pale and generalized body swelling and significant generalized lymphadenopathy. Blood pressure was high and oxygen saturation was under 88%. She was diagnosed with nephritic syndrome possibly secondary to a lymphoma. However, ascitic fluid cytology was negative for malignant cells. At day 16, she passed away. On autopsy it was revealed marked enlargement of the axillary, cervical, hilar mediastinal, para-aortic and mesenteric lymph node groups. Microscopic examination of these lymph nodes showed intracellular and extracellular haematoxylinophilic bodies, each surrounded by a small halo, corresponding to *Histoplasma* characteristics that, based on their size, were classified as *Histoplasma capsulatum* [22].

In 2014, Chatterjee and col reported two autopsy cases of fibrosing mediastinitis with dominant cardiac involvement due to *Aspergillus*. A man of 29 years from North India presented intermittent and low grade fever for 3 months and occasional rigor. He developed shortness of breath on exertion associated with productive cough and occasional hemoptysis. A tomography of the chest showed a homogenous plaque-like soft tissue mass encasing the heart, inseparable from pericardium and walls of cardiac chambers. The mass was also encasing the tracheobronchial tree, pulmonary arteries and entire aorta. No necrosis and calcification was noted. The clinical diagnosis was rheumatic heart disease with a mediastinal tumor. The patient was administered antibiotics, but died before a biopsy was performed. In autopsy, it was identified a firm-to-hard infiltrative and fibrous lesion. Microscopy showed fungal profiles consistent with morphology of *Aspergillus* spp. DNA sequencing identified the species as *Aspergillus flavus* [23].

In a case of acquired haemophagocytic lymphohistiocytosis due to coccidioidomycosis, Ramsi and col described in 2014 a case of a 13-year-old healthy boy from Central California that presented into the hospital with a 7-day history of influenza-like symptoms, fever, abdominal and back pain that failed treatment with antibiotics. He was febrile, tachycardic, profoundly hypotensive and in respiratory distress. Aggressive resuscitation was initiated, but he progressed to decompensated shock, with cardiovascular collapse and respiratory failure, requiring intubation and inotropic support. He was started with broad spectrum antimicrobial medications. His family history was of a sibling who had cocciodioidomycosis manifested by fever, cough and isolated lung nodule and a positive serology test. Given his state, he was transferred to the authors institution for initiation

of extracorporeal membrane oxygenation. His laboratory exams were altered and indicated pancytopenia and acute renal failure. Chest X-ray demonstrated diffuse patchy opacities throughout both lung fields and bilateral pleural effusions. Echocardiogram showed underfilled, hyperdynamic left ventricle with well-preserved function and no structural abnormalities. Abdominal CT scan was remarkable for splenomegaly, and the brain CT scan was negative for acute intracranial pathology. A bone marrow aspirate performed on hospital day 2 demonstrated haemophagocytosis. The infectious workup was unremarkable, and unable to identify an infectious aetiology. Blood, urine, respiratory and bone marrow culture samples were all sterile, and the anti-streptolysin O and anti-DNAse B were negative. HIV, EBV, herpes simplex and other viruses serology were negative. Serum coccidioidomycosis was also negative. Due to his progressive multi-organ failure, haematophagocytic lymphohistiocytosis was established and he received treatment for bacterial causes of this syndrome, but he died on the third day after admission. Autopsy findings were significant for active pulmonary coccidioidiomycosis, extensive pulmonary haematophagocytosis and marked bone marrow histiocytosis. Final diagnosed was of haemophagocytic lymphohistiocytosis due to coccidioidomycosis infection [24].

Parikh and col in 2015, reported a 70 year-old male was admitted into a hospital with abdomen pain and inability to pass stool for 8 days. He didn't show any signs of fever, nausea, vomiting and change in urine or faeces colour and didn't have history of tuberculosis, diabetes mellitus, hypertension or bronchial asthma. Despite a tender and distended abdomen, his vitals and blood tests were normal. Microscopy of the small intestine during autopsy showed features of gangrene with perforation, superadded infection and the presence of slender septate, acutely branched fungal filaments of *Aspergillus* spp., which were further confirmed with special stain of Gomori Methanamine Silver (GMS) [25].

In 2015, Yuan and Prayson reported a case of a 55 year old woman that presented new onset headaches behind the left eye one year prior to death. She developed blurred vision and scotoma 3 months later. After a left temporal artery biopsy was negative for giant cell arteritis, she received a trial treatment of indomethacin. Magnetic resonance imaging (MRI) showed enhancement and thickening of the left optic nerve. Because of the loss of vision and a positive antinuclear antibody test, she was given high dose of steroids. Although it improved her headache, it didn't improve her sight. Screening for infectious diseases including fungal infections such as *Aspergillus* failed to demonstrate an infectious aetiology. Shortly before death, she developed right-sided weakness and

evidence of a pontine infarct on MRI studies. After autopsy, histologic sections taken from the left optic nerve showed an abscess and evidence of fungal hyphae morphologically consistent with *Aspergillus* spp.[26].

A fatal case of disseminated histoplasmosis was reported by Bonsignore and col in 2017 of a 43 year-old floriculturist Italian woman. She was in good health and good medical history and serology. All blood tests were normal. Albeit being splenectomized before, she was considered immunocompetent. The patient died 3 days after admission, 35 days after symptoms onset. Histopathological studies were conducted and indicated disseminated histoplasmosis with multivisceral involvement. Post-mortem blood culture findings confirmed positivity for the pathogen. A multivisceral infiltrate of histiocyte-macrophage cells was evident. The cytoplasm of these phagocytes was laden with black granules, whose morphology was consistent with *Histoplasma.* Lung specimens showed microfoci of confluent, cheese-like necrosis with sero-fibrinous exudate and a weak surrounding inflammatory response; Grocott staining identified fungal hyphae and spores indicative of complicating *Candida albicans* infection [27].

DISCUSSION

Amongst the cases presented, *Aspergillus* was the main cause of death. These findings match what Saini and col described in 2010. In their study, they reviewed neuroimaging features of craniocebral aspergillosis infection in immunocompetent patients. They searched the database of medical records on their institute for fungal infections of the brain and craniofacial area. Of the 22 patients found to have fungal infection in a 12-year period, 12 were of immunocompetent hosts with aspergillosis [28].

The 1-3 β D glucan was tested only in two cases [20,21], indicating that fungal infections weren't primarily suspected due to the immunocompetent state of the hosts and that it is an important tool to diagnose these mycoses. Obayashi and col published in 2008 a study where they evaluated the effectiveness of the 1-3 β D glucan test for diagnosis of invasive fungal infections. They studied autopsy cases from 6 years that had 1-3 β D glucan tested within 2 weeks before death and concluded that this test is an effective diagnostic tool for invasive fungal infections [29].

Antimicrobial treatment was initiated before microbiological assessment was performed, misleading the course of treatment to antibacterial instead of antifungal agents. In these cases, Gram stain is recommended to verify which type of microorganism is causing the infection. If

identified as bacterium, its corresponding treatment can be followed. Yoshimura and col (2017) researched the impact of Gram stain results on initial treatment selection in patients with ventilator-associated pneumonia and concluded that antimicrobial treatment based on Gram stain results restrict the administration of broad-spectrum antibiotics without risk of treatment failure [30].

Autopsy gave the definite diagnosis in 10 of the cases presented. It's important to notice the value of these findings, for not only these subjects wouldn't have the right cause of death determined, but also because it's a matter of public health. In 2012, Colombo and col assessed the importance of necropsy studies to provide data on aetiology, underlying diseases and risk factors associated with fungal diseases, that are essential to improve treatment protocols [31]. Knoke, Bernhardt and Schwesinger (2008) concluded in their review that autopsy remains an important tool for quality control in medical diagnostic and therapeutic activity and that only an exact death statistic makes specific health care possible [32].

CONCLUSIONS

Invasive fungal infections in immunocompetent hosts are rare, especially those with fatal disclosures. In most cases, mycoses in these subjects are treatable and cured as they have full immune responses. Most cases of invasive fungal infections, especially fatal ones, occur in immunocompromised hosts. Although uncommon, mycoses can have deadly outcomes in immunocompetents as well.

Since invasive fungal infections are most commonly found in immunocompromised patients, these diseases are difficult to diagnose in immunocompetent, for they are not expected in this group and main diagnosis is often based on clinical signs, leading to error in diagnostic. Definite diagnoses occur mostly after autopsy. Proper and early mycoses diagnosis is necessary so survival rates are higher within this population.

Data like profession, age and local of residency are important to trace a profile of the patients that have each fungus, so that, in the moment of hospital admission, the physician can have solid indications of which mycosis the patients present and he will be able to give the proper treatment for the individual.

Gram stain and 1-3 β D glucan tests are essential before starting antimicrobial therapy, for they can direct the course of treatment whether to antibiotic or antifungal agents and they can be carried out in almost any hospital or emergency centre worldwide, for they are cheap, simple and easy to

perform. When in life diagnosis is not possible, it is fundamental to have autopsies performed to aid in the profiling of infections aetiologies.

In this review study, *Aspergillus* spp. was the most present fungus in mycoses cases in immunocompetent individuals, highlighting the need of attention in the search for the correct diagnosis since this fungus is very present in the environment.

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ANEXO A - Normas da formatação da revista "World Journal of Pharmaceutical and Medical Research"

INSTRUCTIONS TO AUTHORS

World Journal of Pharmaceutical and Medical Research (WJPMR) is a **Bimonthly Research** Journal which publishes original research articles, review articles, case reports, short communications in the field of Pharmaceutical, Medical and Biological sciences.

WJPMR allows free unlimited access to abstract and full-text. The journal focuses on rapid publication with facilities of, online research article tracking & Email/SMS alert etc. Manuscripts are accepted for review with the understanding that same work has not been published, that it is not under consideration for publication elsewhere, and that its submission for publication has been approved by all authors and institution where the actual work was carried out.

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SUBMISSION OF THE MANUSCRIPT

All submissions must be in the English and should be submitted by the online submission system. Each manuscript will be provided with a manuscript ID and all correspondence is done through e-mail. Please always refer to the manuscript ID for any further enquiries. Authors are invited to suggest up to three potential reviewers, together with their contact details (e-mail addresses are essential). In case of any problem through the online submission, submit manuscripts to our mail: editor.wjpmr@gmail.com or editor@wjpmr.com along with a covering letter preferably by the corresponding author.

MANUSCRIPT STRUCTURE

RESEARCH ARTICLE FORMAT

The preferred format of all manuscripts are in MS office (2003 or above). Manuscript should be concisely typewritten in 1.5 spaces in A4 sized sheets. The pages shall be numbers consequently. Only on one side, with a 1″ margin on all sides. The manuscript shall be prepared in Times New Roman using a font size of 12 and title shall be font size of 14, bold space capitals. All section titles in the manuscript shall be in font size 12, bold face capitals and subtitles in each section shall be in font size 12, bold face lower case. Illustrations (Figures & Tables) must be inserted at appropriate place in the article. Standard International Units should be used throughout the text. Pages should be numbered properly. There shall not be decorative borders anywhere in the text including the title page. The manuscript should be starting with the title page and the text should be arranged in the following order:

- Title Page
- Abstract
- Keywords
- Introduction
- Materials and Methods
- Results and Discussion
- Conclusion
- Acknowledgements
- References

Title Page

The title should be as short as possible on the first page and provide precise information about the contents. The title should be followed by full names of author (s), affiliations of author (s) and institutional addresses.

Authors and Co-Authors Details and Their Affiliations

Each author must provide their full name including their forenames and surname. The Corresponding Author of the manuscript must be marked with an asterisk*, and should be listed

first. In addition the corresponding author must include Telephone and E-mail address at the bottom left corner of the title page. If any of the co-authors are from different organizations, their addresses too should be mentioned and indicated using numbers after their names. Maximum 6 authors should be allowed.

Abstract

Provide on a separate page an abstract of not more than150-250 words. A concise and factual abstract is required. The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, standard nomenclature should be used and abbreviations and references should be avoided.

Keywords

Provide three to six appropriate keywords after the abstract.

Materials and Methods

It should be complete enough to allow experiments to be reproduced. All the procedures should be described in detail, previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results

Results and their significance should be presented clearly and concisely, preferably in the form of graphs and tables which should be self explanatory.

Discussion

It should contain a critical review of the results in the light of relevant literature. Results and Discussion may be combined.

Conclusions

This should state clearly the main conclusions of the research and give a clear explanation of their importance and relevance. Summary illustrations may be included.

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Journal references

1. Cantarelli MA, Pellerano RG, Marchevsky EJ, Camina JM. (Title of article). Anal Sci, 2011; 27(1): 73-8.
2. Sather BC, Forbes JJ, Starck DJ, Rovers JP. (Title of article). J Am Pharm Assoc, 2007; 47(1): 82-5.

Books

- Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B and Kinzler KW (eds.). The Genetic Basis of Human Cancer, New York; McGraw-Hill: 2002, pp. 93-113.
- Bard AJ, Faulkner LR. Electrochemical Methods: Fundamentals and Applications. 2nd ed., New York; John Wiley and Sons: 2001.

Patents

- Aviv H, Friedman D, Bar-Ilan A, Vered M. US Patent, US 5496811, 1996.

Websites

- Database of Natural Matrix Reference Materials, Compilation prepared by International Atomic Energy Agency (IAEA), http://www.iaea.org/programmes/nahunet/e4/nmrm/browse.htm/. For other types of citation, please see "Uniform Requirement for Manuscripts Submitted to Biomedical Journals: Sample References" at www.nlm.nih.gov/bsd/uniform_requirements.html

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