From Aspergillus to Zygomycetes: Invasion of the Fungi

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It sounds like a scene out of a horror movie: Tiny spores of rot-eating fungi, finely dispersed in the air, are inhaled and reach the pulmonary alveoli. There, they mature into fungus mycelium, decompose the lungs and keep growing in the bloodstream, to beset other vital inner organs and destroy them. Yet, systemic fungal infections are a real risk in many medical fields. The course of the disease is serious and any delay in its treatment increases the mortality. Which is already high: Depending on the fungus, the health status of the patient and the concomitant treatment, it lies between 30 and 90 percent.

Aspergillus fumigatus has emerged as the most frequent cause of invasive fungal infections in Europe.
© Dr. David Midgley

Thanks to medical progress, healing prognoses may continuously improve even for the critically ill. However, the complex interventions into the human physiology, especially with intensive medical treatments, also lead to an ever increasing number of patients being threatened by invasive fungal infections.

The Risk of Intensive Care

One of the causes first in line is the artificial suppression of the immune system. Introduced for transplantation patients and people with autoimmune diseases, this treatment is now applied with a growing group of patients - for example after operations or critical burns. But also intensive care itself, including the preventive distribution of broad-spectrum antibiotics, intravenous catheter and feeding tubes, increase the risk of fungal invasion. The basic principle is: The longer the stay in intensive care units, the more likely a systemic fungal infection becomes.

Moulds play an important role in biodegradation. In immunocompromised humans they cause severe infections with poor outcomes.
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Furthermore, certain disease patterns with limited immune response also increase the risk of a systemic fungal infection: AIDS, poorly controlled diabetes or lung diseases like e.g. COPD (chronic obstructive pulmonary disease), to name only a few.

A particular risk factor is neutropenia. That's what medical doctors call the decrease in certain white blood cells, the neutrophile granulocytes. When the number of neutrophiles falls below a certain value, a fungal infection becomes likelier from day to day. About 20 percent of all patients with neutropenia fall ill with a fungal infection. A decrease in these important immune cells is also an undesired side effect of many curative treatments, e.g. with chemotherapy.

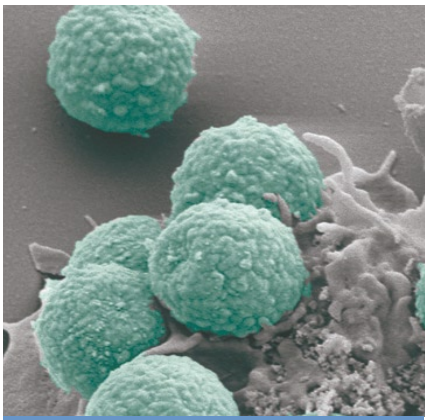
Enemies of the immunosuppressed state

Fungi count as one of the less virulent among the known pathogens. However, they are typical opportunists and take advantage of every possible gap in the body's immune defence. While at first it mainly concerned patients in haematology and oncology, whose immune systems were weakened by the disease itself or due to the chemo or radiation therapy, patient numbers have continuously increased worldwide for the last 30 years in many medical fields.

„Improvements in medical care have resulted in critically ill patients surviving longer, rendering them vulnerable to invasive fungal diseases" (Lass-Flörl 2009). © Pavel Losevsky / Fotolia.com

Between 1970 and 1990, it was above all *Candida albicans* that patients with weakened immune systems feared. 70 percent of the population harbour the yeast as part of their natural skin flora. Controlled by the intact immune system, competing bacteria and the physical barriers of epithelia, it usually remains harmless. If a factor in the protective system alters though, an increased growth of *C. albicans* first leads to superficial wounds on the skin and mucosa. If the immunity weakness is extreme however, the unicellular organism is even able to break through the protecting epithelial barriers by forming hyphae und reach the bloodstream.

And even mould, that usually only decomposes dead tissue, can cause systemic infection in people with extreme immunity weakness. The most frequent pathogen is the omnipresent mould *Aspergillus fumigatus*, which grows visibly as a green coating on fouling foods or invisibly in moist substrates like potting soil. Daily, we breathe in hundreds of its few micrometer-long spores (conidia), that are tiny enough to manage to reach the end of the respiratory tracts, the alveoli.



Conidia of A. fumigatus are constantly inhaled. In healthy humans, however, they are immediately killed by phagocytosis and reactive oxygen species. © Manfred Rohde, Matthias Gunzer /HZI

An intact immune system has no trouble dealing with these unwelcome intruders. In the case of immune weakness though, the conidia find in the lung alveoli an environment that they love: warm, moist and nutritious. Without the body resisting, one single conidium is already enough to form destructive fungal mycelia. "Invasive aspergillosis" is what medical doctors call it, when conidia germinate the lungs, thus form hyphae, to cross the bloodstream borders and finally spread throughout the whole body.

Diagnosis Dilemma

The symptoms of invasive fungal infections are at first nonspecific and difficult to separate from a bacterial infection: fever, decreased blood pressure, raised pulse. Doctors routinely prescribe antibiotics for such harbingers of a life-threatening sepsis ("blood poisoning") because they often think of the much more common bacterial infection first. With severe consequences, because fungi don't react to antibiotics. On the contrary: Broadband antibiosis counts as a systemic fungal disease risk factor. And any delay in starting a treatment fitted to the pathogen and basic disease leads to a dramatic worsening of the prognosis.

An early diagnosis however is rare. A particular difficulty is distinguishing between a mere external fungal settling and an invasive infection. What's more is that not all fungi can be observed microbiologically. For instance, only about 50 percent of invasive candida infections can be proven, and with mould the hit ratio is even lower.

Serological methods aim at detecting fungal-typical polysaccharides from the cell wall, like galactomannan and 1,3-beta-glucan, or show specific antibodies in the blood. But not a single of these tests provide reliable results. In addition, the types of basic disease and treatment or even the nutrition influence the test results.

Without a reliable diagnosis, a treatment always remains empirical, which means that antifungal drugs are prescribed based on experience until the patient's health improves - or not. On many other occasions, doctors give high-risk patients antimycotic drugs prophylactically. With conflicting success: There is no doubt that the lives of many patients have been saved this way. Yet other fungus types also benefit, taking advantage of the now open loophole.

Shift in the Pathogen Spectrum

Because where *Candida albicans* can effectively be fought, other candida types are now on their way. Among the successful non-albicans candidas (NAC) are i.a. *C. krusei* and *C. glabrata*. A Danish study reports a significantly higher percentage of NAC isolates that show lower sensitivity to the prophylactic drug.

Yeasts of the genus Candida are common inhabitants of the normal human flora. © GrahamColm

Mould also benefits. The preventive treatment with a prophylactic drug approved for *Candida-albicans* infections even counts as a risk factor for a systemic infestation by zygomycetes. These fungi of the genera *Mucor*, *Rhizopus* and *Rhizomucor* are even more aggressive than *Aspergillus*. The course of the disease is fast, serious and the prognosis bad. According to the SEIFEM-2004 study, patients with haematological illnesses now suffer from systemic infections more often than from candida invasions.

Among patients with neutropenia, the currently rare but highly deadly infections with the mould Fusarium are especially feared. The fungi cause skin lesions and thus penetrate deep into the body or infect respiratory tracts. The worst of outcomes are brain abscesses and

the spread throughout the entire body. Mortality lies at up to 90 percent. The situation is further aggravated by the fact that the available antimycotics have little impact on these fungi. Data and samples of systemic infections with these and other rare fungi are being collected and documented since 2006 by the European network Fungiscope.

Pathogenicity Factors and Potential Targets

Fungi are like eukaryotes. Because of the similarity of their hosts' cell physiology they present only few target points for pharmaceuticals. More targets and maybe also more effective targets for interventions can possibly be revealed by global analyses of host-pathogen interactions. 'Cause what's for sure: The immune weakness of the host may be a prerequisite, but the fungi also actively contribute to penetrating the inside and can easily adapt to the altered situation.

For instance, *C. albicans* shows a distinctly different transcription profile when changing from harmless yeast into aggressive hyphae. That way, it can not only adapt to the modified living conditions, but can also actively manipulate epithelial cells. Today, a number of genes are known that help *C. albicans* with their invasion. Such genome-wide analyses can contribute to finding intervention points for as many *Candida* types as possible.

Mushrooms, yeasts and other fungi share special features like cellwalls made of 1,3-beta-glucan. © Karl Dichtler / pixelio.de

A. fumigatus is also of the rather adaptable type when entering the bloodstream. For instance, it has a regulative protein in its repertoire that enables survival in oxygen-poor milieus. For further growth in the inhospitable host, the fungus expresses, as a stress reaction, a protein phosphatase named calcineurin. Studies with immunosuppressed mice have shown that the fungus is no longer virulent after a mutation of the respective gene. The competition comes from a different repository: Cyclosporin A, currently used in hospitals as an immunosuppressant, is a fungi-derived peptide and, as calcineurin-antagonist, impedes the growth of *A. fumigatus.* The combination with another active substance (caspofungin) reinforces the action to a fungicidal effect.

Meanwhile, different transnational programmes are researching the fungal pathogenicity factors and their genetic foundations. If they succeed in manipulating them with active substances, the fungal invaders could be rendered harmless for humans, the researchers hope.

Effective medication is urgently needed. Of today's applied antimycotics, only few target a broad spectrum of fungus types and have a high efficiency rate along with reasonable side effects. A blatant gap, because systemic fungal infections have long stopped being a rarity.

Improvements of echinocandin antifungal agents (e.g. Caspofungin) show to be promising - aiming at fungi-specific targets, as well as the possibility of passive immunization against fungal antigens based

on the model of smallpox vaccination. In this case, applications against *C. albicans* are already on their way to the clinical trials phase.

Think of Fungi

The treatment of fungal infections is expensive. Avoiding them or treating them as efficiently as possible, is therefore an imperative for profitability in health care. Given the deficits in diagnostics and the few effective broad spectrum substances for treatment, this isn't easy. Nevertheless - for the last ten years, standardised guidelines on the assessment of invasive mycoses have existed. They were initiated in 2002 by the *Mycosis Study Group of the European Organization for Research and Treatment of Cancer* and have since been further refined.

This raises awareness of the possibility of a fungal infection among doctors. Since certain patient groups have different fungal enemies, a doctor can evaluate a fungal invasion as "possible", "probable" and "proven", based on patient factors and clinical symptoms, whereby "proven" is a relatively rare evaluation. By combining this with microbiological and imaging techniques of fungal detection, diagnosis and hence treatment choice are to be facilitated.

Currently, the research project RESYME is examining to what extent these guidelines are applied. The joint project of the German Society for Mycology (DMykG) and the Department for antimycotic chemotherapy of the Paul-Ehrlich Society (PEG) documents the current application of antimycotic therapies, as well as data on the epidemiological situation in Germany. Because not only effective drugs are relevant for the treatment of such serious infections like those by fungi, but so is their rational application.

Rapid and reliable identification of fungal invasion is still challenging. Besides culture based tests and imaging procedures new diagnostic techniques are being developed. © Mustafa Mohamed Alikhan

References and additional reading:

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Web links

- http://www.aspergillus.org.uk/
  Information on Aspergillus infektions for scientists, doctors and patients
- http://www.dmykg.de/
  Information on the medical and veterinary mycology of the German-speaking „Mykologische Gesellschaft e.V."
- http://www.doctorfungus.net/
  The Official Website of the Mycoses Study Group
- http://www.eucast.org/
  The European Committee on Antimicrobial Susceptibility Testing. Documentation
- http://www.fungiscope.net
  Systemic infektions with rare fungi are currently being documented by European Fungiscope. This global register has been collecting data and samples since 2006.

- http://www.isham.org
  International Society for Human and Animal Mycoses (ISHAM)

- http://www.manasp.org
  Development of novel management strategies for invasive aspergillosis

- http://www.pathogenomics-era.net/projects
  Overview of genome research on human pathogen micro-organisms (ERA-NET), an initiative of the German Federal Ministry for Education and Research