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Contents

The challenge of invasive fungal infection in immunocompromised patients

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Infectious complications are a major cause of morbidity and mortality in immunocompromised patients. With improved control of bacterial infection, invasive fungal infections have become an important issue for these patients.

Immunosuppressive therapies with cytotoxic drugs or steroid, the presence of graft-versus-host disease in transplantation, surgical procedures, widespread use of implanted devices, and administration of broad-spectrum antibiotics have dramatically increased the incidence of fungal infections in this group of patients.

Changing epidemiology of fungal infection
Since the 1950s and 1960s and until recently, the key opportunistic fungal pathogens were *Candida albicans and Aspergillus fumigatus*.

While infections due to *Candida albicans* and invasive aspergillosis remain the most common invasive fungal infections, many centres report an increased prevalence of non-albicans candidal infections. These non-albicans candida species include *C. glabrata, C. guilliermondii, C. krusei, C. lusitaniae, C. parapsilosis, C. pseudotropicalis, C. rugosa, C. stellatoidea* and *C. tropicalis.* Other invasive fungal infections such as fusariosis and phaeohyphomycosis are also on the rise.

Invasive *candida* infection
The emergence of *Candida* infections due to non-albicans species is important to practising clinicians, because candidal isolates resistant to azole therapy have appeared. *C tropicalis* has been shown to cause invasive disease and disseminated infection. *C krusei* is problematic because it exhibits intrinsic resistance to fluconazole and may have reduced susceptibility to other therapies.

Localised manifestations of candidal infection, such as mucocutaneous candidiasis, esophagitis, and urinary tract infection (candiduria), are not life-threatening. However, presence of Candida at any site is a risk factor for potentially lethal disseminated or systemic candidiasis. Presence of the organism does not necessarily mean infection. The host defence must also be impaired in some fashion. Typical additional risk factors are use of central venous catheters, use of broad-spectrum antibiotics, diabetes, and dialysis.

Diagnosing disseminated candidiasis is unfortunately not straightforward. The single best tool is the blood culture, but this tool has limited value. Even in patients with severe neutropenia or immunosuppression, blood cultures are positive only 50% of the time.
There are some additional clues that may be useful in selected patients. Nodular cutaneous lesions may occur in very immunocompromised patients with candidemia, and clearly indicate that bloodstream invasion and dissemination have already occurred. Biopsy of the lesions would confirm the diagnosis. Radiographic imaging, including CT scanning or MRI, is also useful in diagnosing disseminated lesions on the lung, liver, spleen, or kidney, although the radiographic findings may not be pathognomonic.

Aspergillosis
Invasive primary *Aspergillus* infection is an important cause of mortality among bone marrow transplant recipients. The most common pathogenic species is *A. fumigatus*.

Aspergillosis manifests quite differently from *Candida*. Localised disease appears as cutaneous lesions, sinusitis, tracheobronchitis, and aspergilloma (a bronchopulmonary granuloma). Sino-orbital disease can progress to fatal cerebral abscess.

Aspergillus is present in the environment, and the portal of entry is most commonly the lungs. The organism has a predilection for vascular tissue, and often invades blood vessels to the extent that the supplied organ is infarcted. Radiologic findings in neutropenic patients with pulmonary aspergillosis include pleural wedge-shaped lesions, nodular densities, cavitary lesions, necrosis and diffuse bilateral infiltrates. A "halo" lesion is sometimes seen on lung CT and is strongly suggestive of tissue necrosis due to an angiocentric mould. Blood cultures from patients with disseminated *Aspergillus* infection are rarely positive. Biopsy, culture, and histologic examination of tissue reveal the organism.

Emerging fungal pathogens
Fusarium
It is the most prevalent plant fungus world-wide, and it is now recognised as a human pathogen as well. *Fusarium* species are found in the air and soil throughout the world. A variety of *Fusarium* species have been recognised as human pathogens including *F. solani, F. moniliforme, F. oxysporum*, and *F. dimerum*. There is an increasing frequency of *Fusarium* infection in neutropenic patients.

Some species produce mycotoxins and when ingested cause gastrointestinal disease. Continued ingestion of the toxin can lead to aplastic anemia and death. Most systemic infections involve the lung or sinuses, and occur in severely immunocompromised patients such as bone marrow transplant recipients and patients with acute leukaemia.

Like *Aspergillus* spp., *Fusarium* spp. has a tendency to invade blood vessels causing thrombosis and infarction.

About 75% of infections occurring in neutropenic patients disseminate. Multiple skin lesions are common in patients with disseminated fusariosis. It can manifest with fever and large ulcerative cutaneous lesions that progress to necrosis, which manifests as sharply demarcated black eschars surrounded by grayish halo. Organs involved in disseminated fusariosis include the lungs, liver, kidney, spleen and brain. In some patients the infection progresses very rapidly. Infection is life-threatening and associated with a poor prognosis.

Zygomycetes
The most common causative agent is *Rhizopus*, a common bread mould that lives on any organic material. Other pathogens include *Mucor, Rhizomucor*, and *Absidia*.

The severely immunocompromised patient may become infected with Zygomycetes via respiratory inhalation. Clinical manifestations may be rhinocerebral, pulmonary, cutaneous, gastrointestinal, or neurologic. The disease manifests with vascular invasion and tissue necrosis, forming black eschar.

Paecilomyces
Deep invasive infection due to *Paecilomyces lilacinus* now occurs more frequently in immunocompromised patients. The disease manifests with skin lesions.
Phaeohyphomycosis
Phaeohyphomycosis is an infection that manifests as a chronic, tissue-destroying, sinus-forming process. As the infection usually follows traumatic inoculation, the most common site for disease is an extremity, most often the feet. Phaeohyphomycosis can be caused by a variety of fungal genera, including *Bipolaris, Exserohilum* (which grows on grass), and *Exophiala*, all of which are darkly pigmented fungi. The infection is usually treatable with surgical resection of the lesion and antifungal therapy.

Pseudallescheria boydii
Pseudallescheria boydii is a mould that lives in the soil and in vegetation and is an agent of mycetoma, a subcutaneous infection. In immunosuppressed patients, it causes pseudallescheriasis, a soft-tissue and pulmonary disease that resembles aspergillosis (clinically and histologically) and can spread haematogenously.

Trichosporon
Trichosporon beigelii is fungus that lives on hair shafts of the scalp, body, and pubic hair as part of the normal flora, and can cause a superficial fungal infection. The prevalence of haematogenous trichosporonosis is increasing among neutropenic patients. Disseminated disease manifests as multiple, erythematous papular or purpural skin lesions.

The presenting symptoms of disseminated trichosporonosis depend upon the predominant site of infection. It can present as acute renal failure, primary pneumonia, maculopapular or nodular skin lesions which ulcerate. It can involve liver and spleen, giving rise to a picture similar to disseminated candidiasis. Systemic *Trichosporon* infection is often fatal with a mortality rate of about 70%.

T. beigelii is usually resistant to amphotericin B in vitro and azoles are preferable therapeutic agents.

Blastoschizomyces capitatus

Blastoschizomyces Capitatus is widely distributed in air, soil and decaying fruit. The majority of *B. capitatus* infections are disseminated and occur in patients with acute leukaemia. The liver is frequently infected, as is the central nervous system, manifested as meningitis or brain abscess causing neurological defecits. Disseminated infection is associated with maculopapular skin lesions in about 30% of cases. The mortality rates in neutropenic patients with disseminated infection caused by this yeast is about 70%.

Penicillium marneffei
Penicillium marneffei is an environmental dimorphic fungi that can cause serious, life-threatening infections in immunosuppressed patients. It has gained particular attention during the AIDS pandemic. It is unclear whether the reservoir is the soil or bamboo rats that are colonised by this fungus. Although localised infections may occur, the majority of cases in immunocompromised patients are disseminated. Fever is present in virtually all patients and initially may be the only evidence of infection. Other common signs are weight loss, skin lesions, anemia, pneumonia, cough, lymphadenopathy and hepatomegaly.

Skin lesions occur in the majority of cases and usually involve the forehead, trunk, arms and abdomen. About 75% of skin involvement is manifested by a generalised papular rash. Ulcers or papular lesions may also involve the oropharynx, conjunctiva and genitalia.

P. marneffei can be isolated from blood cultures in 75% of infected AIDS patients. The organisms have been isolated from bone marrow, respiratory secretions and other infected tissues. *P. marneffei* characteristically infects mononuclear cells.

P. marneffei is highly susceptible in vitro to azoles except fluconazole and moderately susceptible to amphotericin B.

Cryptococcus neoformans
This yeast-like fungus has a world-wide distribution and appears to be spread by birds, especially pigeons, in their droppings. It is one of the micro-organisms occasionally encountered in the AIDS patient. The spores gain entry into the body through the respiratory tract, where they elicit a granulomatous reaction. However, pulmonary symptoms are uncommon; meningitis is the usual mode of presentation. Lung cavitation, hilar lymphadenopathy, pleural effusions and occasionally pulmonary fibrosis occur. Other sites of involvement include skin and bones.

Histoplasmosis and coccidioidomycosis
These infections are also well-recognised complications of HIV infection in the USA where they are endemic in soil. The commonest presentation is pulmonary abnormalities though extra-pulmonary involvement can occur.

The importance of early diagnosis
The mortality rate in neutropenic patients with fungal infections is high. Among neutropenic patients with *Aspergillus* infection, it can exceed 90%. Neutropenic patients with *Fusarium* or *Trichosporon* infection have a mortality rate approaching 100%. Even candidemia is associated with a mortality rate of 50%. Mortality is higher when the diagnosis of fungal infection is not made early.

The challenge of diagnosis
Up till now, there have been no rapid, accurate diagnostic tests that can confirm with certainty the presence of invasive fungal disease. Unless the clinician considers fungal disease early, disease can progress rapidly while the patient is treated aggressively with broad-spectrum antibiotics.

Standard microbiology is often adequate to provide a diagnosis. Concerning tissue biopsies, fungal cultures are not always positive in the presence of invasive disease. Moreover, positive cultures in the tissue do not definitively signify invasive disease; they may represent colonisation.

Radiographic imaging can be useful in certain situations. X-ray and CT can assist in identifying early *Aspergillus* infection. Pulmonary aspergillosis may manifest as focal areas of patchy consolidation, pulmonary nodules, cavitary lesions, a crescent air sign, or a "halo" on computed tomography. A halo sign, an area of low attenuation (increased density), surrounding a nodular pulmonary lesion in a neutropenic or bone marrow transplant patient is highly suggestive of aspergillosis.

Recently, there is a growing interest in diagnostic methods based on detection of fungal antigens and fungal DNA which may assist in making an earlier diagnosis. Rapid assays included antigen-based tests such as the galactomannan (GM) assay for diagnosis of infection due to Aspergillus spp., the mannan assay for diagnosis of invasive candidal infections, and the beta-D-glucan (BDG), a "pan-fungal" assay; as well as PCR-based assays that detect fungal DNA and assays that detect fungal metabolites (such as the D-arabinitol assay).

At present, biopsy of the lesion, culture, and histologic examination remain important components in the work-up and diagnosis of fungal infection. However, the immediate future will likely be a combination of antigen tests utilised prospectively on a weekly basis, with PCR-based techniques available for species-specific confirmation especially in the heavily immunosuppressed patients such as bone marrow transplant recipients.

Current agents for invasive fungal infection
Treatment of deep fungal infections may need to be started empirically, since obtaining the diagnosis can be difficult and often is delayed. Amphotericin B remains a drug of first choice in many settings, although it may not always be effective for *Aspergillus* infections in highly immunosuppressed patients. The triazoles are

first-line and second-line agents for a variety of infections, but emerging resistance may limit their role. Newer agents include lipid amphotericin B, new azoles and candins.

Current Treatment Recommendations for Selected Deep Fungal Infections

Fungal Pathogen	First-line Agent(s)	Second-line Agent(s)
Candida	Amphotericin B, fluconazole	Itraconazole
Aspergillus	Amphotericin B, Itraconazole	
Zygomycetes	Amphotericin B	Itraconazole
Fusarium	Amphotericin B	Itraconazole
Phaeohyphomycosis	Itraconazole	
Trichosporon	Fluconazole	
Penicillium marneffei	Itraconazole	
Cryptococcus neoformans	Amphotericin B + flucytosine	Fluconazole

Lipid formulations of amphotericin B

The parent drug of lipid amphotericin formulations is amphotericin B. Amphotericin B is an intravenous drug with a very narrow therapeutic index. Three lipid amphotericin formulations are now available and have a greatly improved toxicity profile. They are

1. liposomal amphotericin,
2. amphotericin B colloidal dispersion (ABCD),
3. amphotericin B lipid complex (ABLC).

Only liposomal amphotericin is a true liposome. All of them are generally less nephrotoxic than amphotericin B. Nonetheless they can still cause increased creatinine levels and electrolyte disturbances.

Recent studies showed that these lipid preparations demonstrated comparable efficacy and survival rates among neutropenic patients compared with conventional amphotericin B as empirical antifungal therapy. Patients who received the liposomal drug had significantly fewer infusion-related reactions, such as fever and chills or rigor. Nephrotoxicity was also significantly lower.

Unfortunately, lipid formulations of Amphotericin B are expensive. Their use should be limited to patients with impaired renal function or who develop significant renal impairment during conventional amphotericin B therapy and for patients who require prolonged therapy for diseases like aspergillosis.

Itraconazole in cyclodextrin

Itraconazole is a triazole that has long been available as a capsule and, more recently, as a solution. It has a wide spectrum of activity with extensive efficacy and safety data gathered. It is no more effective than amphotericin B for *Candida*.

Oral absorption of the capsule is variable, but now a liquid formulation is available that confers 30% greater bioavailability. This formulation, a solution in cyclodextrin, should now be administered preferentially to the capsule for all immunocompromised patients with systemic fungal disease. Cyclodextrin is a ring of glucose molecules that stabilises the azole drug and increases its absorption, resulting in improved blood and tissue levels of itraconazole.

The clinical utility of itraconazole has previously been limited by lack of a parenteral formulation. An intravenous formulation of the drug, also solubilized in cyclodextrin, has become available that offers improved absorption and serum drug levels, compared with oral preparation.

New azoles

A second generation of triazoles are currently in development. They will offer a broader spectrum of activity against many species of the key genera, compared with currently available triazoles. In particular, they are active against *C. krusei*, which is resistant to fluconazole.

Voriconazole is a second-generation congener of fluconazole active against a wide variety of candidal species. Voriconazole may have more potent activity than fluconazole against some *Candida* species, including *C krusei*. Voriconazole has also demonstrated in vitro activity against *Aspergillus* species resistant to amphotericin B, as well as against a variety of filamentous fungi. The drug has high oral bioavailability and is metabolised by liver. Drug interactions include increasing the effect of prednisone by 34%, increasing blood levels of cyclosporine by 70% and rifampin greatly increases the metabolism of voriconazole. It will be available for twice-daily dosing by oral or intravenous administration.

Other new azoles in advanced clinical trials include ravuconazole (also a second-generation fluconazole-based drug) and posaconazole (a congener of itraconazole) They have a broad-spectrum of antifungal activity, and in particular are active against a wide spectrum of candidal species.

Candins

A new class of drugs, the candins, is being developed to treat fungal infections. They inhibit fungal cell wall synthesis. They exert their action by non-competitive inhibition of (1,3)-beta-D-glucan synthase, which is not present in mammalian cells. Candin agents in development include the pneumocandin caspofungin (MK-991) and the echinocandins LY-303, 366 and FK-463. All the candin agents are fungicidal against all species of *Candida*, and they are also fungistatic against *Aspergillus*.

The pneumocandin caspofungin (MK-991) is the agent furthest along in development. It is active against *Candida* species and *Aspergillus* species, and was effective in vitro against azole-resistant candidal strains.

Preliminary data from clinical trials showed that MK-991 exerts excellent activity for candidal esophagitis, with response rates of approximately 85% (compared with 67% for IV amphotericin). The agent was not active in vitro against *Fusarium*, *Rhizopus*, or *Paecilomyces*. Because it has poor oral bioavailability, it must be given parenterally. The agent is currently in late phase II and III clinical studies.

Immunomodulation
The human recombinant growth factors, G-CSF and GM-CSF reduce severity and duration of neutropenia following intensive cancer chemotherapy. Many in vitro and animal studies have shown that both agents enhance the activity of neutrophils (GM-CSF also enhances the activity of monocytes and macrophages) against various fungi. Interferon-gamma also enhances the activity of neutrophils.

Conclusion
Fungal infection is a major threat to immunocompromised patients. Some progress has been made in developing reliable diagnostic tests. But the difficulty in diagnoses of invasive candidiasis and aspergillosis quickly remains a major obstacle to their successful management. Fluconazole and itraconazole have enhanced therapeutic options but additional agents with broad-spectrum activity are needed. Several new agents are currently undergoing clinical investigation. Cytokine research is expanding rapidly and hopefully will result in the discovery of agents that can enhance the efficacy of antifungal therapy.

Perinatal group B streptococcal infection

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Introduction
Perinatal group B streptococcal (GBS) infection is a known cause of pregnancy complications, maternal morbidity and mortality and neonatal morbidity and mortality. Neonatal infection can be of early onset and late onset (and late late onset). Early onset neonatal infection occurs in 1-2/1000 livebirths, usually acquired by vertical transmission, with its onset occurring in-utero or within 7 days of life (median 1 hour of life). It contributes to about 80% of neonatal group B streptococcal infection. The estimated cost of GBS infection is US$726 million in the USA in 1985 (The Institute of the National Academy of Sciences). However, it is obstetrically preventable.

Pathogenesis of GBS infection and maternal colonisation
About 70% infants are colonised with GBS of the same serotype as the mother and 1-2% will develop GBS infection. GBS can cross even intact membranes and 40% lethal GBS infection occurs this way (Katz & Bowes, 1988). The fetus swallows or inhales the bacteria which produces potent toxins leading to marked inflammatory reactions affecting the fetal lungs and heart and systemic shock.

The critical factor in determining the occurrence of neonatal infection is the maternal serum antibody concentration to GBS. Placental transfer of antibodies, which occurs at about 32-34 weeks, overrides other factors predisposing to neonatal infection (Baker, 1997).

Maternal GBS colonisation is associated with factors like certain ethnic groups, certain geographical areas, multiple sexual partners, lower educational class, increasing maternal age, maternal diabetes, maternal blood group B and concurrent candida infection (Katz, 1993). The maternal colonisation rate is highest in USA (20-40%) and lowest in Japan (3%) and Israel (2-5%). The maternal colonisation in Hong Kong was estimated to be 19% in the 1980' s (Liang et al. 1986).

GBS tends to colonise the lower digestive tract more constantly while it colonises the genital tract intermittently. The former acts as a reservoir. Therefore an ano-rectal swab is mandatory in antenatal screening for GBS if performed and selective media is required for GBS culture (Katz, 1993). Colonisation of genital tract predisposes to neonatal and maternal morbidity and mortality, such as, premature labour, preterm prelabour rupture of membranes, mid-trimester abortion, stillbirth, urinary tract infection (cystitis and pyelonephritis), chorioamnionitis, endometritis (20-33%), endocarditis (in diabetic women), meningitis and even fatal septicaemia with multiorgan failure. Women with colonisation of urinary tract are also more prone to prelabour rupture of membranes, preterm labour and fetal intrauterine death.

Management of women with GBS colonisation
Antenatal treatment of women with GBS colonisation has not been proved to be of value. Intrapartum antibiotics has been shown to be effective in decreasing genital colonisation, neonatal colonisation and neonatal bacteraemia, and hence the need for neonatal treatment.

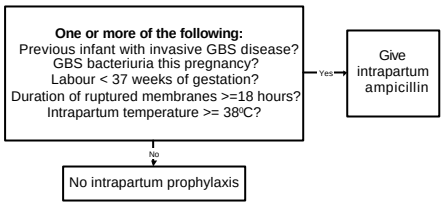
The data from Princess Margaret Hospital provided evidence to support intrapartum prophylaxis. In the period 1996-97, 160 mothers with positive GBS genital tract colonisation delivered in PMH. It was observed that intrapartum antibiotics reduced neonatal colonisation from 64 to 12%.

Screening for GBS colonisation
To identify the women at risk of having babies with GBS infection and to provide intrapartum prophylaxis, various methods of screening have been proposed, including prenatal culture screening, intrapartum rapid tests and screening with risk factors.

Prenatal culture screening have been done at different parts of gestation by various authors but it has been shown to have the best correlation with GBS colonisation in labour if it is done within 5 weeks of delivery (Boyer et al., 1983). Prenatal culture screening identifies about 80-90% of GBS carriers (Bobitt et al. 1985, Boyer, 1988). A negative screen for maternal colonisation is highly predictive of absence of risk to the infants. A positive screen, on the other hand, is low in prediction of neonatal disease.

To reduce the high false positive rate of prenatal GBS screening, rapid test for GBS carrier identification in labour has been proposed and investigated. However, it has the problems of cost and latency for result due to the length of incubation period required (Boyer et al., 1981, Tupperainen et al., 1986).

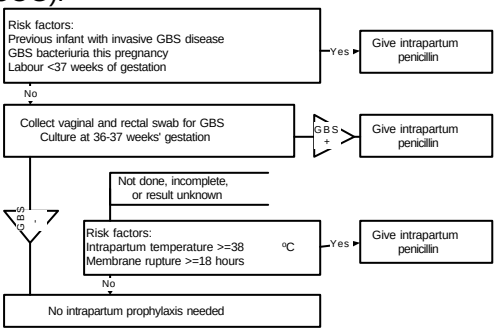
Intrapartum screening with risk factors has a higher positive predictive value at a lesser cost. However it tends to miss the term infants who are not without risk. In fact, nearly 1/3 of GBS infection occurs in full-term infants with no identifiable risk factors for infection (Siegel, 1988). The protocol for intrapartum GBS screening with risk factors is as follows:



The following table shows a comparison of these regimes:

	Prenatal culture screening at 35-37 weeks and intrapartum antibiotics for preterm deliveries and all GBS carriers	Prenatal culture screening at 26-28 weeks; intrapartum antibiotics for GBS carriers who develop intrapartum risk factors	No prenatal cultures; Intrapartum antibiotics for all women with risk factors
Early onset GBS disease prevented	86.0%	50.7%	68.8%
Proportion of women treated	26.7%	3.4%	24.7%

Screening with a combination of prenatal culture and risk factors has been shown to be the most predictive of neonatal GBS disease. Centres for Disease Control and Prevention (1996) has put forward a neonatal GBS prevention strategy combining these 2 methods as follows and this approach has been supported by both American Academy of Paediatrics (AAP) and American College of Obstetricians and Gynaecologists (ACOG):



The choice of screening protocol based on cost-effectiveness will depend on the incidence of neonatal infection in the population. It was estimated that the cost of introducing the risk factor strategy was less than the cost of the disease when the incidence of early onset GBS infection exceeded 0.6/1000 livebirths. Whereas the cost of introducing the screening culture strategy was greater than the cost of disease until the incidence of infection exceeded 1.2/1000 livebirths (Mohle-Boetani et al., 1993). According to a recent study with the retrospective reporting of neonatal GBS infection by questionnaire, the incidence of neonatal GBS infection is estimated to be about 0.5/1000 livebirths in Hong Kong (Dr. B. Lam, 1999).

Intrapartum antibiotic prophylaxis

The recommended antibiotic regimes are: penicillin G 5 million U IV then 2.5 million U IV q4h until delivery or ampicillin 2g IV then 1g IV q4h. If the patient is allergic to penicillin, clindamycin 900mg IV q8h or erythromycin 500mg po q6h can be given. Adequate dosage is required to prevent neonatal colonisation and

infection (Siegel, 1998).
Immunisation appears to be an attractive prevention option in the future though babies born prematurely (who are at risk of GBS infection) are not protected by it because the placental transfer of maternal antibiotics does not occur till 32 to 34 weeks gestation. Problems of licensing and liability need to be solved before its marketing (Boyer & Gotoff, 1988).

Rapid laboratory diagnosis for infections

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Rapid laboratory diagnosis is useful under the following circumstances: (1) when specific therapy for the infectious disease concerned is available or (2) when prompt infection control action is required or (3) when empirical treatment with antimicrobials is considered.

Clinicians should understand the impact that changes in sensitivity, specificity, prevalence of the disease can have on the predictive value of a particular diagnostic test. Sensitivity of a test refers to the rate of positive test when the disease is present whereas specificity refers to the rate of negative test when the disease is absent. Sensitivity and specificity are inherent properties of a given test. Once established, they remain constant and are independent of the population tested. On the other hand, positive and negative predictive values are dependent on both the inherent sensitivity and specificity of the test and the prevalence of the disease among the population tested. Prevalence of disease in the population has more impact on predictive values than sensitivity and specificity of the test. This applies especially to low prevalence area where screening test has to be confirmed by more specific tests.

Microbiological diagnosis can be achieved via organism or patient related technologies. Among organism related technologies, commonly used techniques include direct visualisation, cultivation and detection of antigens or metabolites of the organism concerned. Newer molecular methods are available for detecting and identifying the organism directly in clinical specimen or for identifying culture isolates. Detection of antibodies is a form of patient related technologies.

Rapid diagnosis found its usefulness in a wide array of clinical situations. The following account will focus on some popular examples.

Diagnosis of meningitis
Gram smear and Indian ink examination of the CSF is useful but its sensitivity is related to the concentration of the organism. For example the sensitivity of the technique is less than 25% when the concentration of the organism is less than 1000/ ml. Isolation by cultures remains the gold standard and should always be performed, even in patients already treated with antibiotics. Cultures of organism also provide antibiotics susceptibility testing results. Tests for detection of bacterial antigens by immunologic methods, such as latex particle agglutination, have sensitivities in the range of Gram stain or culture and are of doubtful utility when used routinely, but they can sometimes identify organisms in patients with partially treated bacterial meningitis and negative Gram stain and culture.

Diagnosis of influenza
Direct antigen detection, such as Directigen EIA, can help to confirm the diagnosis of influenza within 1 hour. The test is objective, specific and is more than 90% sensitive when performed on nasopharyngeal aspirate. Compared to conventional and shell vial cultures which require a few days for a result, Directigen EIA is obviously advantageous in terms of rapidity. This is particularly relevant upon the availability of new neuraminidase inhibitors for treatment in the early phase of the illness. PCR targeting at the RNA of the virus can produce result in one day' s time but is technically demanding.

Diagnosis of tuberculosis
Direct visualisation of tubercle bacilli on smear using Ziehl-Neelsen or Auramine O stain is a simple and rapid method for diagnosing tuberculosis (TB). Open TB can be identified and this is important for public health reasons. The shortcoming is that reading smear is labour intensive and microscopist dependent which renders the sensitivity at most, moderate.

Rapid culture using Bactec radiometric system provides an alternative to conventional culture. But still, the turn around time ranges from 2 to 4 weeks as compared to 4 to 6 weeks for conventional culture. However, its sensitivity and specificity is high.

Antigen detection using lipoarabinomannan has been reported as having a sensitivity of 91% and specificity of 100%. This contrasts greatly to antibody detection to detect TB, which gives a sensitivity of 16 to 57% and a specificity of 80 to 97%. Thus the former method looks promising as a future candidate for rapid diagnosis of TB.

Amplicor® Mycobacterium Tuberculosis Test (Amplicor), a nucleic acid amplification (NAA) test, had previously been approved for the direct detection of M. tuberculosis in smear-positive respiratory specimens. Its sensitivity is much lower when used for extrapulmonary specimens (at best 85% compared to 94% for pulmonary specimens). Overall, the positive predictive value of the test when used for pulmonary and extrapulmonary specimens is both 100% whereas the negative predictive value was 96.6% and 96.1% respectively. Though attractive as a diagnostic test, Amplicor is not immune from problem of interference by inhibitors (overall 3.9%) and contamination common to PCR tests. Hence it is important to remember that nucleic acid amplification tests can enhance diagnostic certainty, they do not at this stage replace AFB smear or mycobacterial culture, and certainly not replace sound clinical judgement.

Other systems available on the market e.g. non-radiometric systems and amplified mycobacterium tuberculosis direct test (AMTD) from Gen-Probe, have equivalent performance status.

Other applications
These include detection of MRSA using latex agglutination PBP2a detection, diagnosis of Penicillium marneffei infection by detection of galactomannan by latex agglutination and diagnosis of Respiratory Syncytial Virus using direct immunofluorescence. Prompt antimicrobial therapy and infection control measures are made possible with the advent of these tests.

Conclusion
Each modality of diagnostic method has its inherent advantages and disadvantages. If culture of organism is taken as the golden standard (highest specificity), microscopy, antigen and antibody detection are inferior but the latter methods enjoy the benefits of lower cost and shorter turnaround time. Molecular methods are usually highly sensitive and specific but are costly and involve expertise in set up. Clinicians and microbiologists have to exercise discretion in choosing suitable tests to meet the needs of specific clinical situations.

Case reports and review on typhus

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Typhus is a global health problem, and its public health impact on productivity loss and lives is suspected to be grossly underestimated. Yet, the diagnosis is often missed because of its non-specific clinical features, especially during the early stage of clinical course. We herewith report 2 cases of typhus, following which is a discussion about this important disease.

Case 1
A 26-year-old lady gave 5 days' history of fever and chills, associated with generalized maculopapular rash which appeared 1 day after onset of fever. She also complained of myalgia, arthralgia and profound malaise. She was a hepatitis B carrier. Past health was otherwise unremarkable. No recent travel was noted. However, she visited her mother who lived in a rural village in western part of New Territories regularly, and she needed to traverse an area full of bushes before reaching her mother's home.

Physical examination revealed an eschar over left upper abdomen and generalized discrete maculopapular rash. Pulse-temperature deficit was noticed. Results of preliminary blood tests were unremarkable. Weil-Felix test showed a titre of 1/40 for OX-K, OX 19 and OX-2. She was treated empirically with a course of doxycycline and had an uneventful recovery. Study of paired acute /convalescent sera for rickettsial antibody using indirect fluorescent antibody assay (IFA) demonstrated a greater than 4-fold rise in *Rickettsia conorii* titre.

Case 2
A 52-year-old gentleman with good past health was admitted because of fever for 8 days. This was associated with headache and epigastric discomfort. In addition, generalized maculopapular rash was noted 1 day before admission. He returned from Sarawak for a business trip lasting for 3 days about 2 weeks ago. Physical examination confirmed the finding of rash and revealed tender hepatomegaly. No eschar was identified. Investigations showed elevated ALT with peak level greater than 1000 U/l. Level of OX-19 titre checked on admission was 1/320. The diagnosis of murine typhus was established by subsequent study of serum rickettsial antibody using IFA which showed a titre of greater than 1000 for *Rickettsia mooseri*. He was given a course of doxycycline and recovered without sequel during follow-up.

Discussion
Rickettsiae are obligate intracellular bacterial organisms that are maintained in nature through a cycle that involves arthropod vectors (including mites, ticks, lice, fleas, etc.) and mammal reservoirs. The organisms can be categorized into 3 subgroups: spotted fever group, typhus group and a miscellaneous group that includes exotic organisms like *Coxiella burnetii*. Except for louse-borne typhus, humans are incidental hosts. The arthropods acquire this organism either by transtadial or transovarial transmission. This zoonotic disease is spread to human host by inoculation of arthropod' s faeces through the skin in typhus group rickettsiae and by secretion of infected saliva into the blood pooled at the site of bite for spotted fever group rickettsiae. With the exception of Q fever, the pathogenesis of rickettsial infection is vasculitis caused by the proliferation of organisms in the endothelial lining.

The vector' s activity and chance of encounter between man and the infected vectors are the key determinants of the epidemiology of typhus. Geographical distribution and seasonal incidence vary widely among different types of typhus. Scrub typhus caused by *Orientia tsutsugamushi* is probably the commonest rickettsial infection in Southeast Asia. In Hong Kong, typhus is a notifiable disease and 27 cases were reported in 1999. The peak incidence occurred in late summer and fall.

The incubation period of typhus ranges from 1 to 2 weeks. Most of the patients were unable to recall history of exposure to the arthropod vectors, and risk factor for such an exposure should always be sought. In the endemic area and during the appropriate season, the triad of fever, headache and rash should alert the physicians of the diagnosis of typhus. However, these features are not consistently present and a wide spectrum of other clinical manifestations has been reported. This includes myalgia, malaise, gastrointestinal upset, cough, lymphadenopathy (common in scrub typhus), conjunctivitis, hepatomegaly, splenomegaly, neurological symptoms and signs like nervousness, tremor, deafness, nuchal rigidity, confusion, stupor and seizure. While presence of an eschar is a very helpful sign, this is absent in some forms of rickettsioses including epidemic typhus, murine typhus (as in case 2) and Rocky Mountain spotted fever. Laboratory findings are non-specific and include leucopenia in early phase and leucocytosis in later part of the illness,

non-specific liver enzyme abnormality, hypoproteinaemia, proteinuria, deranged clotting profile (frank disseminated intravascular coagulopathy is rare), electrolyte disturbances, and CSF finding of pleocytosis and elevated protein concentration. The severity of illness varies. Although many patients had an uncomplicated clinical course, a significant proportion of patients was ill enough to require intensive care (10% for murine typhus, for example). Prompt recognition of the diagnosis and timely administration of antirickettsial therapy could markedly reduce the duration of illness and mortality. Predictors of poor outcome include advanced age, prolonged interval before the administration of specific therapy, high leucocyte count, elevated urea and creatinine level, low albumin, sodium, potassium and calcium concentrations. For murine typhus, the presence of haemolytic disease (e.g. G6PD deficiency) was associated with more severe hepatic involvement. A trend towards more severe infection was noted in those patients treated with sulphamethoxazole-trimethoprim. Mortality among hospitalized patients approaches zero with specific therapy. Death is usually due to circulatory collapse, renal failure and respiratory failure.

The early diagnosis of typhus relies heavily on high index of suspicion. Since early administration of specific antirickettsial therapy could prevent severe or potentially fatal infection, treatment should be commenced without delay while waiting for laboratory confirmation. The Weil-Felix test is now regarded as insensitive and non-specific, and its use for confirmation of diagnosis is largely superseded by serologic study. To diagnose a case of typhus using Weil-Felix test, demonstration of a single titre of 1/320 or greater or a four-fold rise in titre is required. For scrub typhus, antibodies against *Proteus* OX-K antigen could be demonstrated in 50% of cases during second week of illness. For other groups of typhus, antibodies against OX-19 might be found. Currently, the most widely used serologic method for diagnosis of typhus is indirect fluorescent antibody test. A four-fold or greater rise of titre in paired acute and convalescent sera is regarded as diagnostic. Other available serologic methods include complement fixation test, latex agglutination test, ELISA and solid phase immunoassay. Apart from serologic methods, other diagnostic techniques have been developed and include immunohistologic demonstration of organisms from relevant tissue specimens and PCR-based methodology. Culture of the organisms is difficult and potentially dangerous.

The preferred antibiotic for treatment of typhus is tetracycline, and chloramphenicol is an acceptable alternative. Other agents that might be effective are fluoroquinolones and new macrolides. The recommended dose of tetracycline is 25-50mg/kg in four divided daily doses, and for doxycycline, 100mg twice daily. The recommended duration of treatment ranges from 3 to 7 days and in general, antimicrobial therapy should be continued for 2 to 3 days after defervescence. There is evidence that single dose doxycycline therapy might be effective. Relapse may occur, especially when duration of therapy is short or single dose therapy is used.

Prevention of typhus is mainly directed towards control of vectors and potential hosts. Wearing of long-sleeved clothes, use of insect repellants and delousing in case of epidemic typhus are simple and effective measures. Vaccine is not available for typhus. While recovery from infection confers long-lasting immunity to re-infection in case of murine typhus, immunity to re-infection is short-lived for scrub typhus due to heterogeneity of strains. Results of studies for chemoprophylaxis using doxycycline are encouraging but preliminary.

Protothecosis

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Case Summary

62 year-old housewife, she was a retired factory worker for 20 years. She presented with a painful swelling of the left thumb for 2 weeks. She had a history of trigger thumb with injection of drug by general practitioner 6-month ago. She has no history of diabetes or allergy but she has 3-year history of hypertension and she was put on antihypertensive.

She complained of tingle and painful sensation over the left thumb about 4 weeks ago with increasing in severity and not responding to antibiotics. Yellowish pus was aspirated out from the swelling and culture yielded an organism called *Prototheca wickerhamii.* She was initially put on ampicillin and cloxacillin but there was still whitish discharge from the wound. After the culture result, intravenous amphotericin B was given and the wound healed well.

Introduction

Protothecae are unicellular, achlorophyllous organisms belonging to the same phylogenic family as green algae of the genus *Chlorella*. Prototheca infection is a relatively rare infection of man and animals. There are over 10 species, but mainly *Prototheca wickerhamii* and *Prototheca zopfii* cause human infection.

The organisms are found in a wide range of environmental sites such as slime flux of trees, collecting systems of domestic or municipal sewage, marine water, and soil. Human protothecosis has been reported in different forms, the most common one is cutaneous and subcutaneous infection, followed by a localized form involving the articular bursa e.g. olecranon bursitis and disseminated diseases occurs in immunocompromised hosts, there are also case report of meningitis, endocarditis and intestinal infection.

Clinical features

- Cutaneous infection
  - the most common reported cases of infection
  - most patients are immunodeficient
  - coincident with trauma and consequent to defects in skin and mucosal surfaces
  - report of cases associate with local injection of steroid
  - some may show no obvious source of infection
  - patients present with a papulonodular or eczematoid eruption and less frequent, cellulitis, verrucous nodules

- the regional lymph nodes are rarely involved
- lesions arise in areas exposed to protothecae usually by traumatic implantation they are usually indolent, developing slowly in a centrifugal manner

- Olecranon bursitis
  - the second most common clinical form of protothecosis
  - presented as mild, slightly tender erythematous induration of the bursa with production of a variable amount of serous fluid
  - patients are not immunodeficient but usually have had penetrating or nonpenetrating trauma to the affected elbow
  - symptoms typically occurs several weeks after the trauma
  - Disseminated protothecosis
  - quite rare, usually occurs in severe immunocompromised patients

Diagnosis

In the wet mount examination, sporangia of various sizes containing sporangiospores (endospores) are seen but no budding or hyphae are produced. They measure 3-30um in diameter and reproduces by internal septation and cleavage which subsequently rupture and release of endospores. So in the histological section a ' morula' form or ' daisy' like organisms can be found which is quite characteristics of the organisms. Further confirmation can be done with Prototheca antigen specific antibody immunofluorescence. They can be grown from the skin biopsy specimen, joint fluid and blood on Sabouraud agar and blood agar in aerobic condition.

Treatment

Because *Prototheca* species have about 4% ergosterol in the cell membrane, amphotericin B is the recommended treatment. There are studies of in vitro susceptibility test, which indicate that only amphotericin B showed persistent algicidal activity. The efficacy of different azoles varies between agents and most showed low algicidal activity but there are reported cases of clinical improvement after administration of itraconazole or fluconazole.