Invasive Fungal Infections: Diagnosis and Treatments in China

Yuping Ran

Department of Dermatology, West China Hospital, Sichuan University

The body location and clinical appearance of fungal infections depends on the fungal virulence, infectious route and host immunological state. The result being that patients with mycoses consult with different clinical departments. The diagnosis of mycoses is based on the detection of fungal elements such as hyphae and/or yeast cells from the involved tissues. Isolation of the fungus is the precondition for species identification and antifungal treatment. To think clinically and to emphasize the mycology is the basic consideration of medical mycology research. Mycologists play a key role in the collaboration between the clinical and laboratory aspects. The clinician always wants to know what the fungus is and how to treatment the mycosis. Fungal pathogens are often stealthy and difficult to detect in infected patients during the early stages of the diseases and this is when therapies would be the most effective. Routine techniques commonly employed in the detection of fungal diseases including microscopic examination, culturing and serology are seriously hampered by lengthy waits of times for results and low accuracy. The clinician may want prophylaxis or to use empirical antifungal treatment to see if it does/does not work. The problem is that some of the patients do not respond to the antifungal treatment, because the doctor lacked sufficient evidence of fungus infection to give the doctor confidence to continue treatment. Accurate and early diagnosis of fungal diseases is critical for managing mycotic diseases. This is usually done by direct microscopic examination (DME) of KOH preparations. Good specimens are the key point that directly affects the quality of microscopic evidence and culture. The most important aspect is culturing samples on different media with or without chloramphenicol and cycloheximide and incubated at room temperature and 37℃. Early treatment could save a patient's life. We start treatment at the time we have the proof of fungal infection, i.e., KOH positive. Itraconazole, fluconazole, terbinafine, amphotericin B or its liposome form, can be used alone or in combination based on the fungal species involved and the site of infection. **[Kor J Med Mycol 2008; 13(3): 121-128]**

Key Words: Direct microscopic examination, Antifungal drug

Introduction

The body location and clinical appearance of fungal infections depends on the fungal virulence, infectious route and host immunological state. The result being that patients with mycoses consult with different clinical departments. The diagnosis of mycoses is based on the detection of fungal elements such as hyphae and/or yeast cells from the involved tissues. Isolation of the fungus is the precondition for species identification and antifungal treatment. To think clinically and to emphasize the mycology is the basic consideration of medical mycology research. Mycologists play a key role in the collaboration between the clinical

†Corresponding author: Yuping Ran, M.D., Ph.D. Department of Dermatology West China Hospital, Sichuan University Chengdu, 610041, P. R.
Tel: +86 13980297369, Fax: +86 13980297369
e-mail: ranyuping@vip.sina.com

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1. Diagnosis

Accurate and early diagnosis of fungal diseases is critical for managing mycotic diseases. Our experience is before starting antifungal treatment, we need to be sure the tissue was invaded by a fungus. This is usually done by direct microscopic examination (DME) of KOH preparations. Good specimens are the key point that directly affects the quality of microscopic evidence and culture. For example, cornea samples should be taken by ophthalmologist and samples from the external ear or vocal areas should be taken under the otoscopy or fiberoptic laryngoscopy by an ENT doctor. Media for fungi culture should be available to isolate all possible fungi in the sample. The most important aspect is culturing samples on different media with or without chloramphenicol and cyclo-heximide and incubated at room temperature and 37℃. Molecular identification, such as ITS1/2

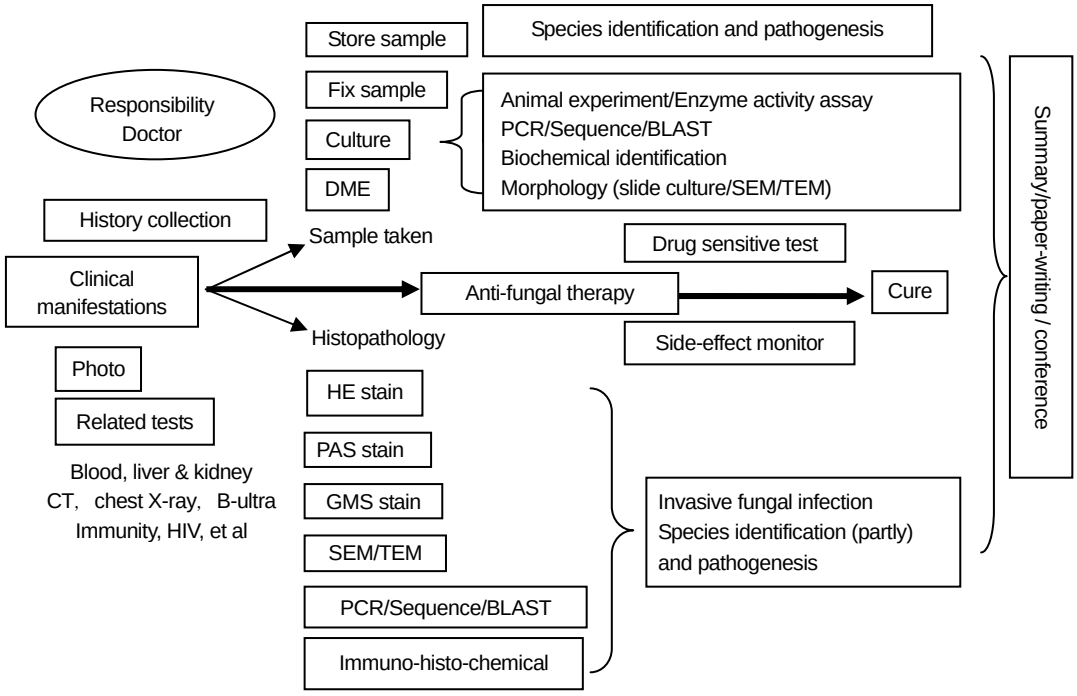

Fig. 1. Scheme for diagnosis and treatment of invasive fungal infections

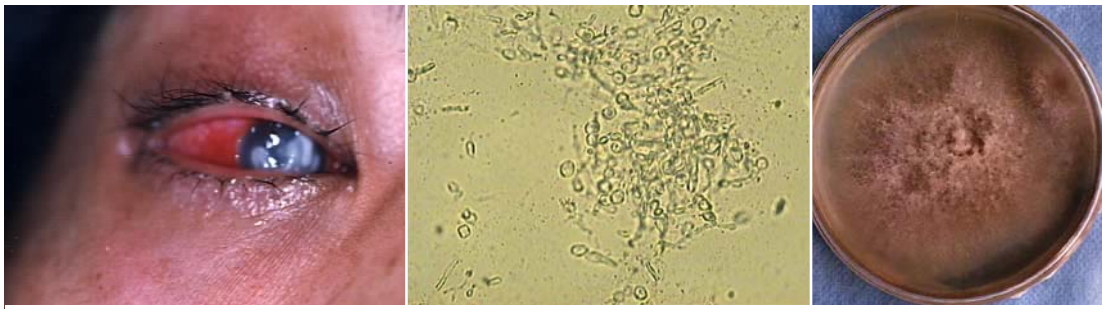

Fig. 2. Clinical picture (right cornea abscessed), pus smear on KOH direct examination (spores & hyphae) & Culture (*Fusarius* was isolated) of a 38-year-old male

based PCR reactions and sequence comparisons with BLAST is very important for publications but requires much work and expense and usually does not help in early treatment.

2. Treatment

Early treatment could save a patient's life. We start treatment at the time we have the proof of fungal infection, i.e., KOH positive. Before giving an antifungal drug, we collect samples for culturing and freezing the samples at -20℃ for repeated culturing since the first culture may be contaminated or failed. Parted samples are fixed with 2% glutaraldehyde for later pathology study and TEM/SEM observation. Itraconazole, fluconazole, terbinafine, amphotericin B or its liposome form, can be used alone or in combination based on the fungal species involved and the site of infection. Drug sensitive testing should be done using the isolate. Be prepared to change drugs when clinical results are not satisfied or the first choice drug induced side-effects.

Based on our experiences in west China, we have encountered the following pathogenic or opportunistic fungal species: *Fusarium sp*., *Microsporum gypseum*, *Malassezia spp*., *Sporothrix schenckii*, *Aspergillus fumigatus*, *A. terreus*, *Trichophyton mentagrophytes*, *Cryptococcus neoforman*, *Candia spp*., *Penicillium marneffei* and mixed infections. These involve organs such as the eye, nose, middle ear, mouth, vocal cord, face, scalp, subcutaneous, bone, lymph nodes and dissemination to numerous internal organs. Some cases are illustrated below, and, listed in references.

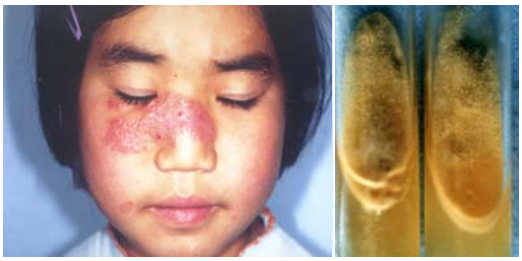

Fig. 3. Clinical picture (dark red plaque developed on right face and nose), Culture (*Mucor* was isolated from the tissue of the biopsy) of a 14-year-old girl

Case 1.

A 38-year-old male peasant. His right cornea abscessed and loses his eyesight after trauma. Spores and hyphae were detected by pus smear on KOH direct examination. *Fusarius* was isolated from the pus (Fig. 2).

Case 2.

A 14-year-old girl. Her right face and nose bridge developed dark red plaque after received a tear-bag washing operation, with out effect by topical and orally application of antibiotics. *Mucor* was isolated from the tissue of the biopsy. She was cured by intravenous of amphotericin B (Fig. 3).

Case 3.

A 4-year-old boy with verrucous hyperplasia

plaque on his right ala nasi was consulted. Light microscope observed the organism after inoculated on PDA microculture at 25℃ for 7 days: hyphae are narrow, septate and branching, with conidio-

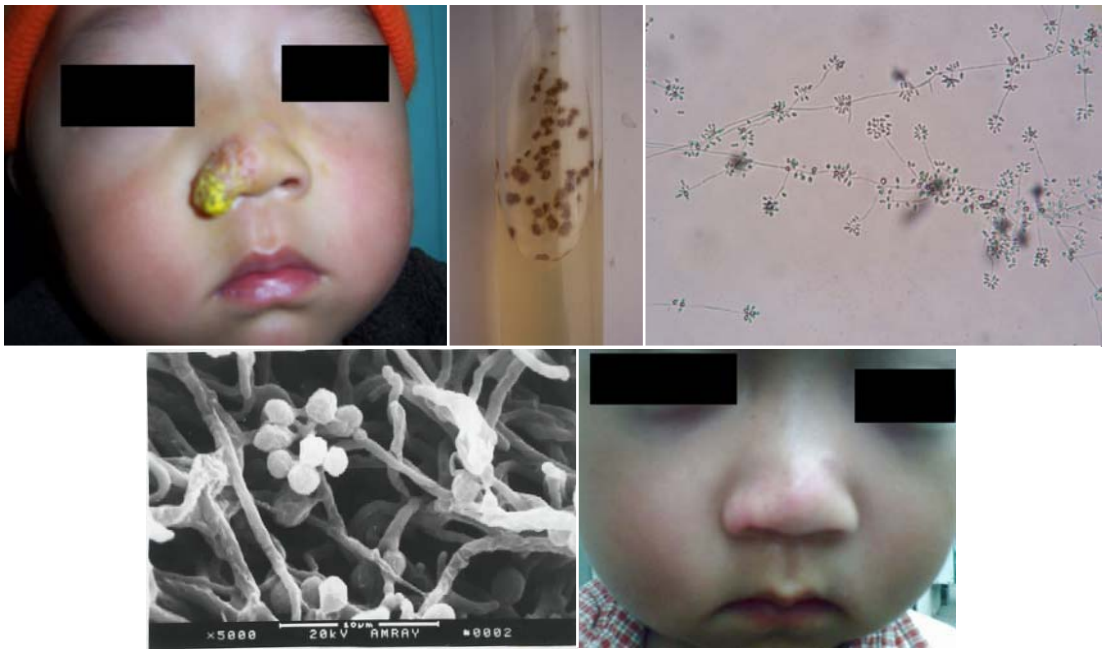

Fig. 4. Clinical picture (verrucous hyperplasia plaque on his right ala nasi), microscope, PDA microculture (hyphae are narrow, septate and branching, with conidiophores rising), successful treatment of a 4-year-old boy

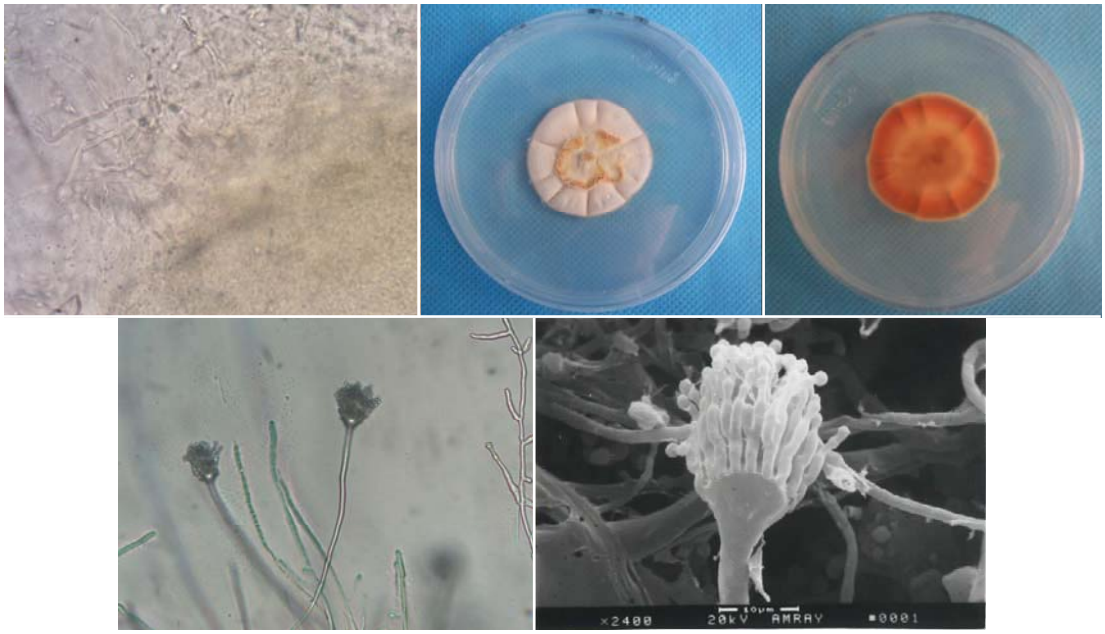

Fig. 5. DME detected of hyphae, conidiophores, and conidial heads in the ear wax, and scanning electron microscope oberseved the organism after inoculated on PDA slide culture (*Aspergillus terreus*)of a 55-year-old man

phores rising at right angles. The ape of the conidiophore bears many oval conidia, forming a "rosette-like" cluster; conidia also develop along the hyphae. The boy was diagnosed as sporotrichosis based on morphology and PCR sequence of the isolate, and, successfully treated by systemic and topical application of terbinafine (Fig. 4).

Case 4.

A 55-year-old man with effusion of both ear canals after trauma, digged his ear wax by toothpick, for more than 2-years was consulted. DME detected of hyphae, conidiophores, and conidial heads in the ear wax. A colony about 3.5 cm in diameter developed after the isolated fungus were incubated on Czapek medium at 28℃ for 7 days. White in surface, floccular in center, flat with radial striates in periphery; yellow to brown from center to periphery of back. Scanning electron microscope oberseved the organism after inoculated on PDA slide culture at 28℃ for 7 days: conidial heads loosely columnar, vesicles subspherical, about 10 μm diam. Conidiogenous cells biseriate, metulae as long as the phialides. Conidia smooth walled, spherical to broadly ellipsoidal. Conidiophore stipes smooth walled and hyaline. This species was identified as *Aspergillus terreus*, based on the morphology and PCR sequence of ITS. The patient was cured by topical drop of fluconazole (Fig. 5).

Case 5.

A 9-year-old boy, who had a history of scalp trauma followed by abscess and ulcer for 28 days. He was administered to a dermatoplasty but abscess recurred 5 days later. Fungal colonies developed on the 4th day after inoculating the pus and broken hairs of the boy's eruption on SDA at 25℃, the colony is white and powdery. Urease test is positive. Grape-like cluster microconidia and spiral hyphae

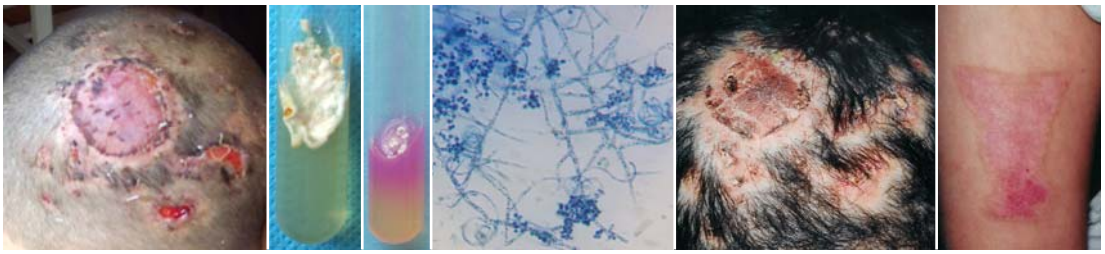

Fig. 6. Clinical picture (abscess and ulcer on the scalp for 28 days after trauma), Urease test (+), SDA Culture of a 9-year-old boy

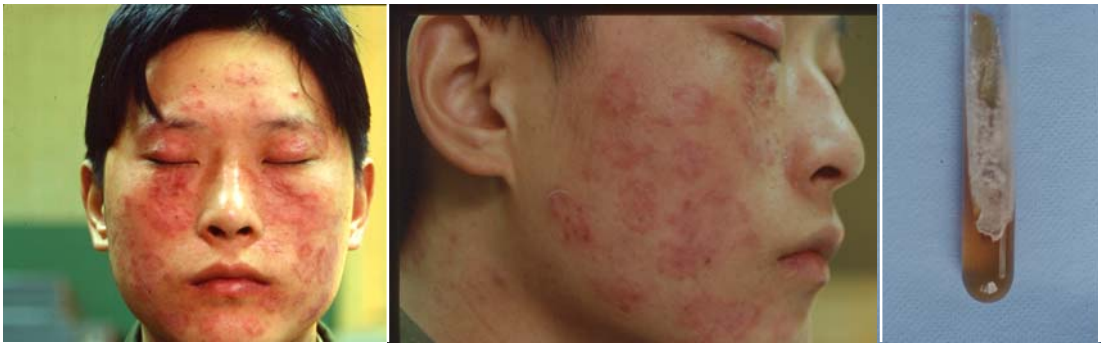

Fig. 7. Clinical picture (erythema symmetrically developed on his face), Culture (*Trichophyton mentagrophytes*) of a 21-year-old veteran

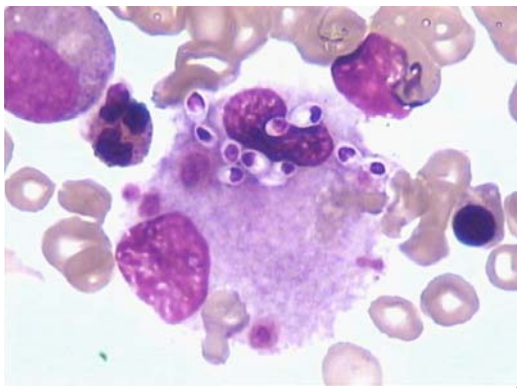

Fig. 8. Bone marrow smear (a lot of budding yeasts inside of the macrophage) of a 68-year-old male

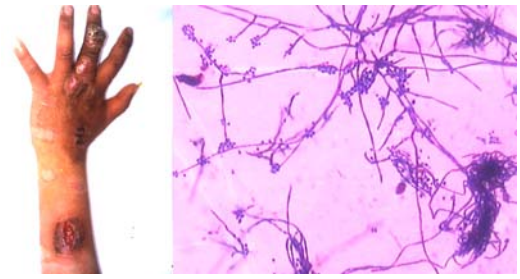

Fig. 9. Clinical picture (a number of nodules developed on his left back of hand and forearm), slide-culture (*Sporothrix schenckii*) of a 9-year-old boy

were seen at the 15th day's slide culture on SDA. The boy's lesion on scalp after being treated with oral itraconazole 100 mg daily (5 mg/kg/d, weight: 22 kg) for 60 days. The area on scalp received skin could not growth of hair for it was from the boy's right thigh, where, superficial scar and pigmentation remained (Fig. 6).

Case 6.

A 21-year-old veteran. He was diagnosed systemic lupus erythematosus (SLE) for the erythema symmetrically developed on his faces in an army clinic. He was hospitalized for plasma exchange treatment without effect. Scales scratched from the facial erythema were cultured and *Trichophyton mentagrophytes* was isolated. He was cured by topical use of antifungal cream (Fig. 7).

Case 7.

A 68-year-old male. He was hospitalized for repeated unknown reason fever, no response to multiple antibiotics treatment. A lot of budding yeasts inside of the macrophage were detected by bone marrow smear. The patient was diagnosed as systemic candidiasis and was cured by systemic antifungal treatment (Fig. 8).

Case 8.

A 9-year-old boy. His left middle figure was stabbed by a plant two months ago and could not heal, and then a number of nodules developed on his left back of hand and forearm even if by antibiotics and repeatedly excision treatment. Bacteria were negative but fungus was isolated when the pus was inoculated on Sabouraud's dextrose agar. Slide-culture was made. Conidiogenous cells arising from undifferentiated hyphae, forming tear-shaped sympodiconidia in groups on small, clustered denticles, which was identified as *Sporothrix schenckii*. The patient was diagnosed sporotrichosis and was cured by orally 10% potassium iodide (Fig. 9).

Case 9.

A 29-year-old female. She was hospitalized in Hemorrhage Department for fever, anemia, and enlargement of liver, spleen and lymph nodes. She had a history of work in Guangzhou, the south of China. Bone marrow smear showed a lot of sausage-like yeasts with septum inside and outside of the macrophage. *Penicillium marneffei* was isolated from her bone marrow. HIV antibody was positive in her blood serum. She was diagnosed as AIDS and *Penicilliosis marneffei* (Fig. 10).

Case 10.

A 33-year-old female. She was hospitalized for multiple nodules and ulcers. A huge ulcer and a sinus developed on her left leg. X-ray showed that the bone of the middle-lower left tibia and the lower left fibula destroyed. Yeast colonies with positive urase grew after cultured the pus, which was identified as *Cryptococcus neoformans*. She

was diagnosed as disseminated cryptococcosis and osteomyelitis. She was cured by systemic application of amphotericin B, fluconazole and itraconazole (Fig. 11).

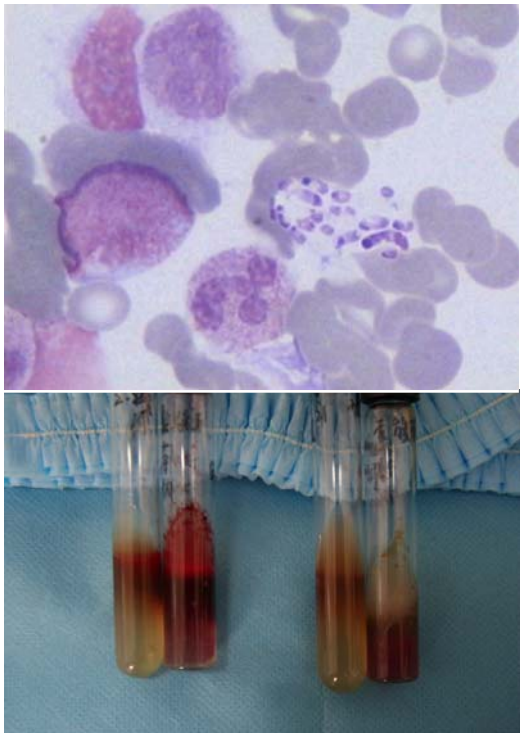

Fig. 10. Culture, bone marrow smear (a lot of sausage-like yeasts with septum inside and outside of the macrophage, *Penicillium marneffei* was isolated) of a 29-years-old female

Conclusion

We should consider the whole picture from the first encounter with the patient to the case report be published. Not every event will be done well for the clinical situation and characters in each case display variety, but a young doctor (or graduate) should be directed to be responsible and especially with the collection information of all of the experimental and clinical data. It is not only good to diagnose and treat the patient systematically, but also this helps to train a young doctor by case study. Cooperation with doctors in different department and technicians in different laboratory is essential, through which a "patient as a center" based cross-academy network could be established effectively.

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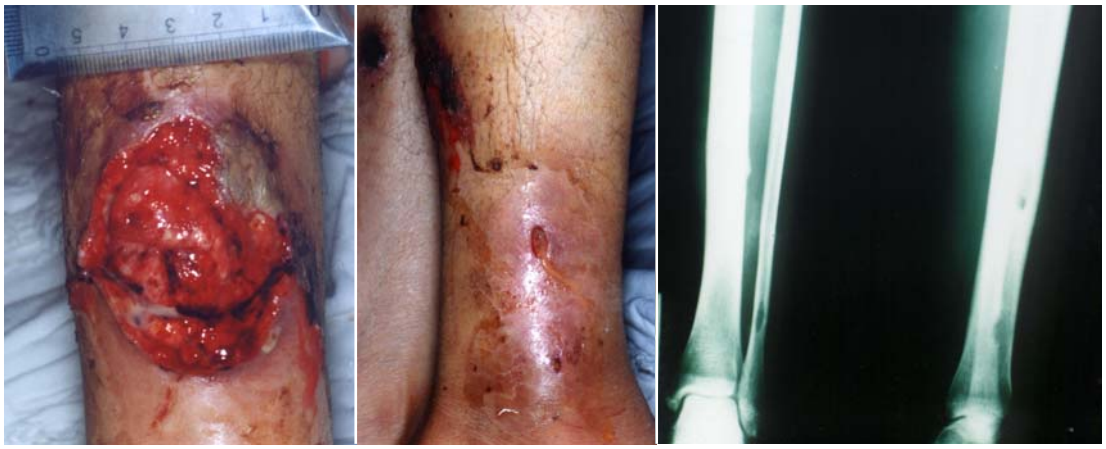

Fig. 11. Clinical pictures (A huge ulcer and a sinus developed on her left leg), X-ray (the bone of the middle-lower left tibia and the lower left fibula destroyed) of a 33-years-old female

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