

3. Hubner NO, Siebert J, Kramer A. Octenidine dihydrochloride, a modern antiseptic for skin, mucous membranes and wounds. *Skin Pharmacol Physiol* 2010; 23: 244-58.

4. Melicherikova V, Urban J, Goroncy-Bermes P. Residual effect of antiseptic substances on human skin. *J Hosp Infect* 2010; 75: 238-9.

5. van Doremalen N, Bushmaker T, Morris DH, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. *N Engl J Med* 2020; 382: 1564-7.

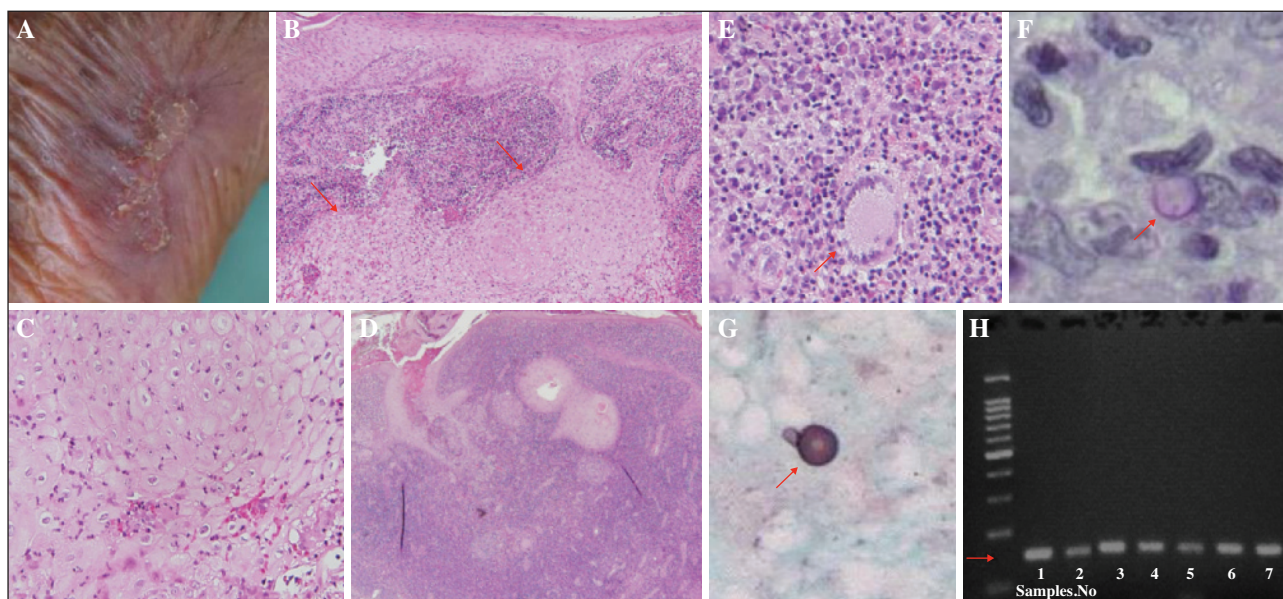
doi:10.1684/ejd.2020.3856

## PCR-based diagnosis of *Sporothrix* infection using DNA from paraffin-embedded skin specimens in previously undiagnosed cases

Definitive diagnosis of dermatomycosis requires identification based on culture of focal eruption samples. However, it is occasionally necessary to also consider culture-negative cases and cases without culture. PCR may aid in the diagnosis of such cases. However, although nested-PCR (involving two rounds of PCR amplification) is highly sensitive, it is liable to contamination, which may affect the interpretation of results [1].

We previously reported identification of *Sporothrix* based on extraction of DNA from formalin-fixed and paraffin-embedded (FFPE) tissues using nested PCR [1]. The sensitivity and specificity of this method was 100% and 98.7%, respectively (sample size: culture-positive  $n=52$ , controls  $n=79$ ). Herein, we present a patient whose biopsy was not submitted for culture because the skin lesion was a suspected cutaneous neoplastic lesion based on clinical and histopathological investigation, however, sporotrichosis was subsequently detected by PCR.

An 85-year-old man was referred to our department because of an irregularly raised, crusted mass on the back of his right hand (*figure 1A*). The lesion was reported to have gradually increased in size over about two months and measured 3.5 cm. A histopathological study of the skin biopsy at the first visit revealed acanthosis, pseudo-cancerous proliferation between epidermis to dermis, and inflammatory cells (lymphocytes, histiocytes, neutrophils, and giant cells) in the dermis (*figure 1B*). Nuclear division was observed in a proportion of the proliferating epidermal cells (*figure 1C*). The clinical and initial pathological findings suggested a highly differentiated squamous cell carcinoma-like lesion. The entire lesion was excised and the skin was surgically grafted. A specimen was not sent for culture. However, histopathological examination of the entire sample showed inflammatory granulation tissue and giant cells, findings suspicious of fungal infection, with no evidence of squamous cell carcinoma (*figure 1D, E*). Staining with Periodic acid-Schiff (PAS) and Grocott's methenamine silver (GMS)



**Figure 1.** A) Clinical presentation of the patient with differential diagnosis of cutaneous squamous cell carcinoma. B-E) Histopathological study of the first skin biopsy (haematoxylin and eosin staining). In the sample from the first biopsy, acanthosis, pseudo-cancerous proliferation between epidermis to dermis (arrow), and inflammatory cells (lymphocytes, histiocytes, neutrophils, and giant cells) are present in the dermis ( $\times 100$ ) (B), and nuclear division is observed in a proportion of the proliferating epidermal cells ( $\times 400$ ) (C). In the entire sample from the second surgery, inflammatory granulation tissue and giant cells (arrow), suggesting a fungal infection, are shown in (D) ( $\times 100$ ) and (E) ( $\times 400$ ). Fungal elements (arrow) are apparent with Periodic acid-Schiff (F; arrow) ( $\times 400$ ) and Grocott's methenamine silver (G; arrow) ( $\times 400$ ). H) Representative PCR-positive results for 7/21 patients (sample No.1 refers to the presented case), whose specimens were not submitted for culture or culture-negative, and who were suspected to have cutaneous sporotrichosis based on histopathological evaluation; the presence of *Sporothrix* is indicated by the presence of the 152-bp fragment (arrow).

showed fungal elements (figure 1F, G). Therefore, our PCR method for *Sporothrix* was performed using the FFPE sample retrieved at the second surgery, and a positive result was obtained. The patient received additional treatment with an antifungal agent for one month after which there was no recurrence.

Cutaneous sporotrichosis is a deep dermatomycosis caused by organisms of the *Sporothrix schenckii* complex [2], and is known to occur frequently on the face of children and extremities of the elderly [3, 4]. The gold standard for diagnosis involves the isolation of the fungus based on a culture test. Because these fungi are not detected superficially on the skin, definitive diagnosis requires culture of skin biopsy samples. However, cultures occasionally return false-negative results [2]. In addition, skin biopsy is invasive, requiring local anaesthesia, and is especially difficult to perform multiple times on the face in children. Moreover, it is occasionally challenging to distinguish these lesions from other infections and cutaneous neoplasms [5], therefore cases exist in which a culture has not been submitted or the disease is not suspected prior to skin biopsy.

We collected 21 biopsy specimens from patients at Dokkyo Medical University (1979 to 2019), who were suspected to have cutaneous sporotrichosis based on histopathological evaluation (haematoxylin and eosin, PAS and GMA), but for whom cultures were not obtained or were negative. PCR of DNA samples obtained from FFPE samples revealed positive results in 81.3% (17/21) of the patients (seven samples are presented in figure 1H).

Other ancillary diagnostic tools for cutaneous sporotrichosis include a sporotrichin skin test, microscopic examination after applying potassium hydroxide and a histopathological test. However, these tests do not always provide positive results [4]. A method for extracting DNA from fresh samples and performing PCR for early diagnosis has also been reported [6, 7], however, in such cases, a suspicion of cutaneous sporotrichosis is required before surgery or skin biopsy in order to obtain a sample for culture testing.

Cutaneous sporotrichosis occasionally relapses [3], in particular, in patients for whom culture was not performed and definitive diagnosis was not made. In these situations, patients require a clinical diagnosis from testable samples, thus the PCR method from FFPE samples could aid diagnosis. However, false positives due to contamination may occur [1], therefore diagnosis should be considered along with comprehensive clinical findings and other tests in addition to PCR. ■

**Disclosure.** *Conflicts of interest: none. Acknowledgements: this study was supported by a grant from Dokkyo Medical University, Young Investigator Award (No.2019-10), Tochigi, Japan. Ethical Statement: informed consent was obtained from the study participants, including consent to participate and publish the findings. The authors confirm that the ethical policies of the journal, as noted in the Declaration of Helsinki, have been adhered to and appropriate ethical review and approval by the institutional review board of Dokkyo Medical University were received.*

<sup>1</sup> Department of Dermatology,  
Dokkyo Medical University  
<sup>2</sup> Division of Clinical Science,  
Dokkyo Medical University, School  
of Medicine  
<shayashi@dokkyomed.ac.jp>

Masami KOIKE<sup>1</sup>  
Shujiro HAYASHI<sup>1</sup>  
Akiko BABA<sup>1</sup>  
Saiko KOIKE<sup>2</sup>  
Takeshi GONMORI<sup>1</sup>  
Tomoko KAMINAGA<sup>1</sup>  
Eisuke ISHII<sup>1</sup>  
Satoko ISHIKAWA<sup>1</sup>  
Yoichiro HAMASAKI<sup>1</sup>  
Ken IGAWA<sup>1</sup>

1. Hayashi S, Kaminaga T, Baba A, *et al.* Diagnostic value of a nested polymerase chain reaction for diagnosing cutaneous sporotrichosis from paraffin-embedded skin tissue. *Mycoses* 2019;62: 1148-53.
2. de Meyer EM, de Beer ZW, Summerbell RC, *et al.* Taxonomy and phylogeny of new wood- and soil-inhabiting *Sporothrix* species in the *Ophiostoma stenoceras-Sporothrix schenckii* complex. *Mycologia* 2008; 100: 647-61.
3. De Lima Barros MB, de Almeida Paes R, Schubach AO. *Sporothrix schenckii* and Sporotrichosis. *Clin Microbiol Rev* 2011; 24: 633-54.
4. Arenas R, Sánchez-Cardenas CD, Ramirez-Hobak L, Ruíz Arriaga LF, Vega Memije ME. Sporotrichosis: from KOH to Molecular Biology. *J Fungi (Basel)* 2018; 4: 62.
5. Rodríguez-Brito S, Camacho E, Mendoza M, Niño-Vega GA. Differential identification of *Sporothrix spp.* and *Leishmania spp.* by conventional PC and qPCR in multiplex format. *Med Mycol* 2015; 53: 22-7.
6. Hu S, Chung WH, Hung S, *et al.* Detection of *Sporothrix schenckii* in clinical samples by a nested PCR assay. *J Clin Microbiol* 2003; 41: 1414-8.
7. Zhang M, Li F, Li R, Gong J, Zhao F. Fast diagnosis of sporotrichosis caused by *Sporothrix globosa*, *Sporothrix schenckii*, and *Sporothrix brasiliensis* based on multiplex real-time PCR. *PLoS Negl Trop Dis* 2019; 13: e0007219.

doi:10.1684/ejd.2020.3862

## A case of systemic lupus erythematosus/systemic sclerosis overlap syndrome successfully treated with belimumab

The pathogenesis of systemic lupus erythematosus (SLE) involves various immune pathways including B cells, innate immunity and T cells [1]. Successful treatments for SLE overlap syndromes are rarely reported [2, 3], but documenting such cases is crucial for the management of the disease. Here we report a case of SLE/systemic sclerosis (Ssc) overlap syndrome in which a long-term successful response to belimumab was demonstrated.

The patient was a 31-year-old Japanese female with SLE/Ssc overlap syndrome. She was diagnosed with SLE at age 18 due to joint pain, a high fever, stomatitis, anaemia, high serum IgG, and serum anti-double-stranded (ds) DNA and anti-Sm antibodies, and had been treated with prednisolone (PSL) at 5 mg/day and azathioprine (AZA) at 50-100 mg/day. In her 20s, she was diagnosed with Ssc based on serum anti-Scl70 antibody level