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<http://dx.doi.org/10.1016/j.jaad.2016.02.1159>

### Subungual debris cytopathology increases sensitivity of fungus detection in onychomycosis



*To the Editor:* Onychodystrophy has a variety of etiologies, including onychomycosis, psoriasis, lichen planus, eczema, trauma, peripheral vascular disease, systemic diseases, aging, and neoplasms. Onychomycosis, which accounts for half of cases, remains difficult to identify by standard mycologic techniques. A confirmed diagnosis is imperative to avoid unnecessary systemic antifungal treatments and potential toxicity.<sup>1,2</sup> Periodic acid–Schiff (PAS) staining of nail plate fragments is generally considered to be more sensitive than potassium hydroxide (KOH) preparation<sup>3</sup> and culture, although less sensitive, and remains an important method for the identification of challenging or treatment-resistant cases. Reported sensitivities and specificities are listed in [Table I](#).

Even though fungal forms can be easily identified in the subungual hyperkeratotic debris,<sup>4,5</sup> the highly friable subungual material is easily dislodged and is not compatible with histologic processing.<sup>6</sup> One report describes the microscopic examination of PAS-stained subungual debris but requires abundant material.<sup>4</sup>

We describe the novel of application liquid-based cytopathology systems (eg, ThinPrep Pap Test used in cervical cancer screening) for the analysis of subungual debris in the diagnosis of onychomycosis. This study was exempt from approval by the institutional review board of the Legacy Research

**Table I.** Sensitivities of PAS, KOH, and culture methods for the diagnosis of onychomycosis

Method (Reference)	Sensitivity
Culture*	49.5%
Culture*	29.4%
Culture <sup>†</sup>	62.1%
Culture <sup>‡</sup>	79.3%
Culture <sup>§</sup>	59.0%
KOH*	55.9%
KOH <sup>§</sup>	80.0%
KOH + Culture*	72.1%
KOH-A <sup>†</sup>	96.7%
KOH-A <sup>‡</sup>	90.9%
KOH-R <sup>†</sup>	83.3%
PAS*	93.1%
PAS*	88.2%
PAS <sup>†</sup>	93.3%
PAS <sup>‡</sup>	98.8%
PAS <sup>§</sup>	92.0%
PAS <sup>  </sup>	85.0%
PAS (present study)	88.0%
PAS + Culture*	94.1%

KOH-A, Potassium hydroxide and light microscopy prepared and read by an attending dermatologist; KOH-R, potassium hydroxide and light microscopy prepared and read by a resident dermatologist.

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Institute, Portland, OR. We reviewed all nail plate clipping specimens submitted between January and March 2009 to 1 dermatopathology laboratory for histologic evaluation for onychomycosis. Specimens were processed using a standard processing protocol and paraffin embedding, from which both hematoxylin and eosin (H&E) and PAS slides were prepared. If a sample was negative on the initial histologic examination, the formalin in which the specimen was submitted was processed in a standard thin-layer cell preparation system (Thermo Cytospin and Cytospin) for adjuvant cytologic identification of fungal forms in the subungual debris. The pathologists reading the cytopathology of subungual debris

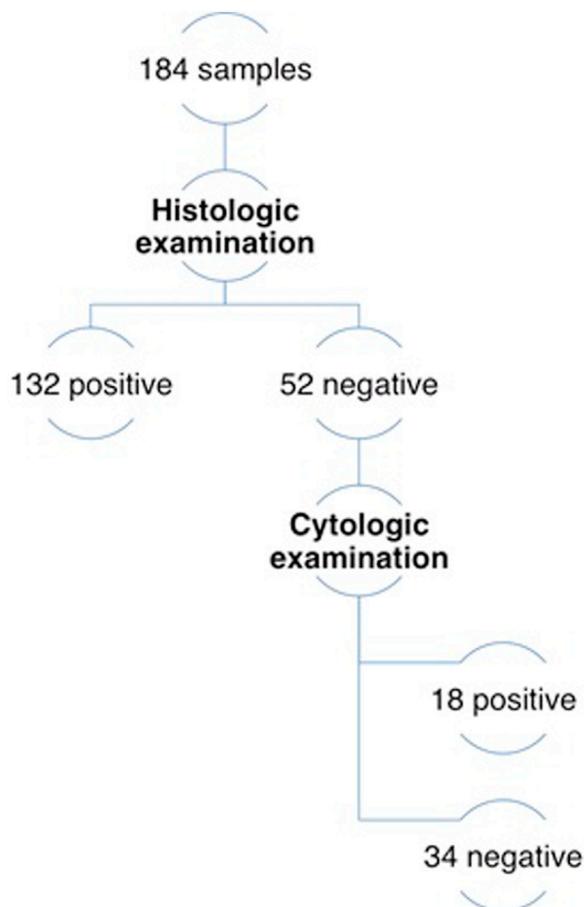


**Fig 1.** Onychomycosis. Microscopic examination of PAS-stained subungual debris. (Original magnification:  $\times 400$ .) Subungual debris was collected by centrifugation of the formalin in which nail clipping specimens were submitted. Microscopic examination of a thin-layer preparation of PAS-stained subungual debris reveals multiple darkly staining fungal forms associated with a single keratin aggregate.

had been blinded with regard to the results of the H&E and PAS stains of the associated nail plates.

Of 184 nail specimens submitted, 150 cases (82%) were diagnosed as onychomycosis and 34 cases (18%) were diagnosed as dystrophic nails. Of the 150 positive cases of onychomycosis, 132 (88%) samples demonstrated fungal forms on the initial histologic examination and 18 (12%) were identified only on subsequent cytologic examination (Figs 1 and 2). An additional set of 11 initially positive samples was tested as control and in all cases the cytologic examination of the subungual debris also demonstrated hyphae; 4 cases had easily identifiable fungal forms, 7 had rare but unequivocal fungal forms, and 1 case had a single form that, although not absolutely definitive, was most consistent with fungus.

Centrifugation of the formalin in which nail specimens are submitted and thin-layer preparation of the resulting subungual debris pellet increases the detection of fungal forms and sensitivity of onychomycosis diagnosis in clinical nail plate specimens; the combination of histologic nail plate examination and adjuvant subungual debris cytopathology is more sensitive than histologic examination of nail plate clippings alone. Clinicians should submit specimens, not only with nail plate clippings, but also with as much subungual debris, in 10% formalin, in order to allow for liquid-based thin layer preparation and cytopathology of subungual debris if necessary. If clinicians submit a nail specimen dry, the laboratory can perform a KOH on the debris or



**Fig 2.** Flowchart of outlining the testing process and results of all clinical specimens. A total of 184 clinical specimens were first processed for histologic examination of the nail plate. Samples that were negative on the histologic examination were processed for cytologic examination of residual subungual debris. Fungal forms were identified in the subungual debris of 35% of the samples (18 of 52) that were negative on preceding histologic examination. The additional 11 samples used as positive controls are not included in this flowchart.

suspend the sample in a liquid, such as formalin, to allow for a thin-layer preparation. Ideally, these preparations should be read by cytopathologists who routinely evaluate similar cytologic preparations.

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Funding sources: None.

Conflicts of interest: None declared.

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<http://dx.doi.org/10.1016/j.jaad.2016.02.1163>

### The impact of secukinumab treatment on the prevalence of human papillomavirus in patients with psoriasis: A pilot study



To the Editor:  $\beta$ -human papillomavirus (HPV) was originally proposed to act as a putative antigen contributing to the pathogenesis of psoriasis.<sup>1</sup> However, research supports the premise that skin in individuals with psoriasis might be more permissive for the presence of HPV than normal skin, and not specifically for HPV5 or HPV36.<sup>1</sup> This study aimed to investigate the impact of secukinumab treatment, an anti-interleukin (IL)-17A monoclonal antibody, on the prevalence of HPV in patients with psoriasis.

This observational and prospective study invited all patients with psoriasis participating in an ERASURE (Efficacy of Response and Safety of Two Fixed Secukinumab Regimens in Psoriasis) trial<sup>2</sup> and its extension study (NCT01544595) in our dermatology clinic between July 2011 and January 2015. Because previous studies had shown a high colonization frequency of HPV in the eyebrow hairs and neck skin, eyebrow hairs and scraping samples from neck skin were collected for HPV detection and analyzed by HPV genechip (EasyChip HPV blot kit, King Car, Taipei, Taiwan)<sup>3</sup> and polymerase chain reactions with Forslund-Antonsson primers<sup>4</sup> at enrollment, week 12, week 24, and week 156 after enrollment in the secukinumab trial.

**Table I.** Characteristics of the study population

Age (years), mean $\pm$ SD	40.3 $\pm$ 10.0
Gender (male/female),	28/4
Duration of psoriasis (years), mean $\pm$ SD	14.4 $\pm$ 6.6
Baseline PASI, mean $\pm$ SD	23.2 $\pm$ 8.6
Accumulated dose of secukinumab treatment (mg), mean $\pm$ SD	7635.9 $\pm$ 3517.7
Accumulated duration of secukinumab treatment (weeks), mean $\pm$ SD	129.6 $\pm$ 41.8
Achieving PASI75 response at week 12	62.5%
Achieving PASI75 response at week 24	78.1%
Achieving PASI75 response at week 52	62.5%
Achieving PASI75 response at week 156	42.9%
Positive HPV DNA in neck skin scrapes at baseline, n	56.3%
Positive HPV DNA in eyebrow hairs at baseline, n	43.8%

PASI, Psoriasis area and severity index.

The demographic features, accumulated treatment dose, and duration of secukinumab treatment in the 32 patients are listed in Table I. A total of 43 HPV genotypes were detected. At baseline, HPV DNA was detected in 43.8% and 56.3% of eyebrow hairs and skin scrapings, respectively.  $\beta$ -HPV was significantly more prevalent (73% to 100%) than non- $\beta$  cutaneous types ( $\alpha$ , 0-20% ;  $\gamma$ , 0-7%) in both eyebrow hairs and neck skin scrapings at baseline, during treatment, and after the treatment. HPV58 was the most prevalent type found in the skin scrapings (11%) and eyebrow hairs (13%). Twelve weeks after secukinumab treatment, the prevalence of HPV DNA decreased to 32.3% and 51.6% in eyebrow hairs and skin scraping samples, respectively. At week 24, the prevalence of HPV DNA in the skin scraping samples was significantly lower than at baseline (56.3% vs 30%,  $P = .039$ ). At week 156, HPV DNA was expressed in a significantly lower percentage of eyebrow hairs and skin scraping samples (20.6% and 20.6%, respectively) compared with baseline ( $P = .013$ ) (Fig 1).

Conversion of HPV status from positivity at baseline to negativity after a 156-week of secukinumab was seen in 12 of 19 (63.2%) patients. Patients who achieved more than psoriasis area and severity index 75 improvement at week 156 had a higher chance of conversion of HPV status from positivity at baseline to negativity at the end of the study ( $P = .045$ ).

Although the mechanism by which anti-IL-17 therapy decreases HPV detection had not been fully understood, research found that elevated IL-17 was associated with inhibited effective host immune