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A new method for in vitro production of *Fonsecaea pedrosoi* fumagoid cells in a synthetic medium

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Introduction: *Fonsecaea pedrosoi* is the principal etiologic agent of chromoblastomycosis, a chronic infection of the skin and subcutaneous tissue. This dimorphic fungus is found in tissues in the form of spherical cells known as sclerotic, Medlar or fumagoid cells. Several methods have been reported to induce the formation of in vitro fumagoid cells based on acidic media supplemented with propranolol or platelet activating factor. However, it is very difficult to achieve the proliferation and growth of these tissue forms, which has hindered the physiologic knowledge of this microorganism in its parasitic phase.

Objective: To evaluate a synthetic medium to produce fumagoid cells.

Methods: Six strains of *Fonsecaea pedrosoi* isolated from human patients with chromoblastomycosis were used. The strains were perpetuated in Sabouraud agar. Subsequently, they were incubated for 5 days under constant agitation (200 rpm) at room temperature in 250 mL Erlenmeyer flask containing 100 mL of Czapek-Dox medium to obtain conidia. Then, conidia were centrifuged at 4000*g* for 10 min at 4°C and washed 3 times in 1 mL PBS. Finally, they were incubated for 3 months at room temperature in the same medium.

Results: At 3 months, abundant round, septated, double-walled, pigmented structures were observed in all strains, compatible with fumagoid cells.

Conclusions: Since it has now been shown that fumagoid cells are the active parasitic forms of *Fonsecaea pedrosoi*, it is necessary to have a simple and easy-to-reach method, which allows obtaining the tissue phase of this pathogen to study thoroughly the host-parasite relationship.

Commercial disclosure: None identified.

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Risk factors and impact of conversion to an indeterminate interferon-gamma release assay result in patients receiving biologics

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Background: Repeated latent tuberculosis infection (LTBI) screening using the QuantiFERON Gold Test (QFT) in patients taking biologics is common practice. QFT results are reported as positive, indeterminate, or negative.

Objective: We evaluated clinical factors and impact associated with conversion from a negative to indeterminate QFT result in patients with chronic inflammatory/autoimmune diseases on biologics at a single tertiary-care center.

Methods: Patients who had >1 indeterminate QFT result while being treated with a biologic (≥3 mo) for autoimmune/chronic inflammatory conditions were identified. Of 479 patients with indeterminate QFT results, 200 patients who converted from baseline negative to indeterminate QFT were identified.

Results: The cohort (n = 200) had a median age of 44 years; 60% were females. The most prevalent risk factor for indeterminate QFT was treatment with steroids (52%) or other immunosuppressants (38%). The majority (97.5%) of indeterminate results were due to low mitogen response. At least 1 risk factor for TB was found in 45% of the cohort. Approximate cost of additional testing following an indeterminate QFT was $320/patient. After indeterminate QFT result, 26 patients (13%) discontinued biologic treatment, but only one was subsequently diagnosed and treated for LTBI. No cases of active TB occurred.

Conclusions: In our cohort of converters to an indeterminate QFT while on biologics, only 0.5% were diagnosed with LTBI. Repeated LTBI screening in patients on biologics in a low-TB-risk population added more harm than benefit when results were indeterminate. Following baseline negative QFT, indeterminate QFT results created confusion, leading to unnecessary costs and delays/discontinuation in beneficial treatment.

Commercial disclosure: None identified.

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How much are patients with acne willing to pay for treatment?

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Background: Almost one-third of patients with acne do not fill their prescriptions, often because of cost. However, the amount patients are willing to pay is not well characterized.

Methods: A survey was administered to participants who reported having acne on Amazon Mechanical Turk, an online crowdsourcing platform. Based on previous studies, our tool asked about willingness to pay (WTP) control (lifelong payment to manage disease), WTP-cure (one-time payment to eliminate disease), and socio-demographic factors.

Results: Sixty-one participants were recruited. The median WTP-cure was $77.50, interquartile range (IQR) $48.75-$212.50. The median WTP-control was up to $50/month. Subjects with incomes below that of the median household had greater WTP-cure as a percent of income than those with incomes above the median (median 0.24% vs. 0.13%, $P < .01$). There was no significant difference in WTP-cure between low-income (median, IQR: $50, $30-$200) and high-income ($100, $50-$225) groups. There was also no significant difference in WTP-control between low-income (up to $50/mo, $50-$75/mo) and high-income (up to $50/mo, $50-$50/mo) groups. Those with private health insurance had higher WTP-cure (up to $100, $100-$500) versus nonprivate health insurance (up to $100, $0-$100) as well as higher WTP-control (up to $50/mo, $50-$100/mo) versus non-private insurance (up to $50/mo, $0-$50/mo), both $P < .02$. Age, gender, and education level did not correlate with either WTP-cure or WTP-control.

Limitations: We did not control for quality of life or acne severity.

Conclusions: Those with private health insurance are willing to pay more for acne medications than those without, regardless of income.

Commercial disclosure: None identified.

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Leveraging CRISPR-Cas12a for the detection of human T-Cell leukemia virus type 1

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Human T-cell leukemia virus type 1 (HTLV-1) is a potent carcinogenic oncovirus that, while asymptomatic in the majority, has the potential to cause devastating complications such as adult T-cell leukemia/lymphoma (ATLL) or HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Despite its high morbidity and global burden, poor access to current testing modalities limits HTLV-1 testing in endemic regions. Here we perform the pre-clinical, proof-of-concept experiments necessary for the development of a sensitive, specific, and low-cost CRISPR-based screening test for HTLV-1. We took advantage of recent reports describing the ability of Cas12a enzymes and guide RNAs (gRNAs) to act as molecular sensors in field-deployable viral diagnostic assays. Upon specific recognition of the gRNA-complementary DNA sequence, eg HTLV-1 proviral DNA, Cas12a/gRNA complexes can catalyze nonspecific trans-cleavage of a single-stranded DNA (ssDNA) reporter, providing an amplified signal of the target recognition event (2). We designed and synthesized multiple HTLV-1-specific Cas12a gRNAs targeted to conserved regions of the HTLV-1 genome. We also designed a custom ssDNA reporter molecule that could be used to measure target-induced Cas12a trans-cleavage activity by quantitative polymerase chain reaction (qPCR). Upon detection of HTLV-1-specific targets in vitro, Cas12/gRNA complexes demonstrated concentration-dependent cleavage of ssDNA. Our assay was able to detect 36 femtomoles of HTLV-1 target DNA and lays the foundation for future efforts to develop a robust, point-of-care CRISPR-based HTLV-1 diagnostic test that could potentially transform the detection and management of HTLV-1 infection.

Commercial disclosure: None identified.