Patients and Methods

Study Design

This study was approved by the ethics committee of Affiliated Shenzhen Longhua People's Hospital of Southern Medical University, Shenzhen, China, and was conducted according to the ethical principles of the Declaration of Helsinki. There is no conflict of interest. None of the authors of the study has financial interests of any type or is in any way affiliated with the manufacturers, wholesalers or retailers of the device under investigation.

I. Optimization of long-pulsed 1,064-nm Nd:YAG laser (LPNYL) treatment of onychomycosis from April 1, 2012, to May 31, 2014.

An orthogonally designed study was conducted (Table 1). If a patient had more than one nail infected by *T. rubrum*, each infected nail was randomly divided into groups A–I and received corresponding laser treatment. Therefore, there was neither a control group nor an experimental group. The first step was merely to determine the optimal treatment regimen (the most effective treatment parameters) of LPNYL for OCTr by multivariate regression analysis.

II. Randomized controlled study of long-pulsed 1,064-nm Nd:YAG laser (LPNYL) treatment of onychomycosis compared with conventional oral antifungal treatment with itraconazole from June 1, 2014, to March 31, 2016.

Patients were randomly divided into the laser group (treated with the optimal regimen and the most effective treatment parameters) or drug group (treated with oral itraconazole). Comparisons of the efficacy and safety of both groups were performed.

Patients

This study was conducted in 246 patients aged 18–60 years who were admitted to the dermatology service from April 1, 2012, to March 31, 2016. All patients with OCTr were determined based on the results of a culture of nail specimens. Patients provided written informed consent and voluntarily accepted follow-up. None had taken external medication within 3 months or systemic antifungal drugs within the previous 12 months.

The following patients were excluded from the study: those who terminated treatment on their own initiative, changed the treatment strategy, or failed to complete timely follow-up; patients who took other drugs that might affect efficacy during the treatment period; patients who persistently or semi-persistently demonstrated nail discoloration; patients who had a history of previous trauma to the target nail,

peripheral arterial disease, diabetes mellitus or peripheral neuropathy of any kind; patients who took a photosensitizer for almost 6 months; patients who were pregnant or lactating; patients who had a subungual hematoma or mole-like tissue; patients whose affected nail was caused by a combination of diseases, such as nail plate psoriasis, lichen planus, and atopic dermatitis; patients with liver or kidney dysfunction; patients who needed to take drugs that cannot be taken in conjunction with itraconazole capsules; and patients who were considered to be ineligible to participate in the trial by clinicians. Treatments terminated due to adverse reactions were not included in the efficacy but were included in the incidence of adverse reactions.

Evaluation of the Onychomycosis Severity Index (OSI)

All researchers participating in the evaluation of the onychomycosis severity index (OSI) did not know to which group the patients belonged. All nails that were studied were evaluated by three dermatologists, excluding the therapist, for their level of severity according to the OSI [Carney C, Tosti A, Daniel R, Scher R, Rich P, DeCoster J, et al: A new classification system for grading the severity of onychomycosis: onychomycosis severity index. Arch Dermatol 2011;147:1277–1282] before and after treatment. No points were given when the entire invasion spot from the entire nail surface on one onychomycosis was 0%, one point was given when the invasion ratio was 1–10%, two points were given when the invasion ratio was 11–25%, three points were given when the invasion ratio was 26–50%, four points were given when the invasion ratio was 51–75%, and five points were given when the invasion ratio was 76–100%. When dividing the nail from the edge to the proximal nail fold into four parts, one point was given for 1/4 or less, two points were given for 1/4–1/2, three points were given for 1/2–3/4, four points were given for 3/4 or more, and five points were given when invasion proceeded to the nail lunula or nail matrix. Thereafter, the two numbers were multiplied, and ten points were added when there was a dermatophytoma or 2 mm or more of subungual hyperkeratosis accompanying the symptom.

Treatment Protocol

Before treatment, the involved nails were clipped to remove as much dystrophic nail as possible without interfering with the nail bed. The nail plates were then buffed manually with an emery board to reduce further disease burden.

For laser therapy, twenty-four hours before starting treatment, all patients were informed about the laser treatment, potential side effects and therapeutic alternatives. Next, they were treated with an LPNYL (Dualis SP; Fotona, Ljubljana, Slovenia). The following parameters were used according to orthogonally

designed table 1: energy density (fluence) of 35, 40, or 45 J/cm^2; spot size of 3, 4, or 5 mm; pulse width (duration) of 25, 30, or 35 ms; and frequency of 1 Hz; treatment times (sessions) of 4, 6, or 8 at 1-week intervals. Regardless of the initial clinical findings, the entire nail plate and lateral and proximal nail walls of all nails (including the nail matrix) were treated. A total of five laser passes (alternating in a two-pass transversal direction, two-pass longitudinal direction, one-pass spiral direction) were administered on each infected nail. A 2-min interval was applied to every infected nail between every two laser passes. If the patient felt pain, a 5-s pause was applied and gentle pressing of the nail by the therapist was performed to reduce the pain. All treatments performed in 1-week intervals were conducted by one dermatologist to ensure consistency. All nails near the infected nails were treated using one-spiral-direction laser pass regardless of whether there was clinical evidence of nail dystrophy.

All patients received a treatment effect evaluation through visits at 6 months and 12 months and did not receive additional treatments for mycosis, such as systemic or external antifungal drugs, during the study period.

For the drug group, the antifungal itraconazole (Xi'an Janssen Pharmaceutical Ltd., China) was administered orally. Each treatment course consisted of 200 mg twice daily for 1 week followed by a 3-week rest. The fingernails were treated with three courses, while the toenails underwent four courses.

During the entire study period, all patients were given information on appropriate hygiene (e.g., wearing cotton socks and loose, flat, breathable, soft-soled shoe; avoiding injury of the nails).

Evaluation of the Clinical Efficacy (CE)

The CE assessment consisted of target nail measurements at 6 months and 12 months. OSI before treatment (OSI_{before}) and at 6 months (OSI_6) and 12 months (OSI_{12}) were calculated. The clearance rate of infected nails = ((OSI_{before} – OSI_6 or OSI_{12}) / OSI_{before})*100%. Cure was defined as a clearance rate >95%. A statistically significant improvement was defined as 75–95%; an improvement was defined as 35–75%; no change was defined as <35%. CE = (number of (cured + statistically significant improvement) nails/total number of infected nails)*100%.

Evaluation of the Mycological Efficacy (ME)

Mycological cultures and microscopy were performed at 6 months and 12 months. A mycological cure was defined by negativity of both culture and microscopy. ME = number of mycological cured nails / total number of infected nails)*100%.

Adverse Effects and Pain

For laser therapy, the treating medical assistant used a survey to record the occurrence of oedema, burning, blisters, (new occurrences of) onychodystrophy, infections, nerve damage or delayed wound healing. Pain during laser treatment was quantified using a visual analogue scale (0 = 'no pain' to 10 = 'most intense pain').

For itraconazole therapy, a complete blood count and liver enzyme levels were measured routinely, and other side effects, such as headache and nausea, were recorded.

Statistical Analysis

SPSS 17.0 software (SPSS, Inc., Chicago, IL, USA) was used for statistical analysis. Analysis of variance, χ^2 test and Newman-Keuls *q*, *t* test, and Fisher's exact probability test were conducted to compare variables, and $p < 0.05$ was considered statistically significant. Multivariate analysis was performed using logistic regression (forward: Wald).