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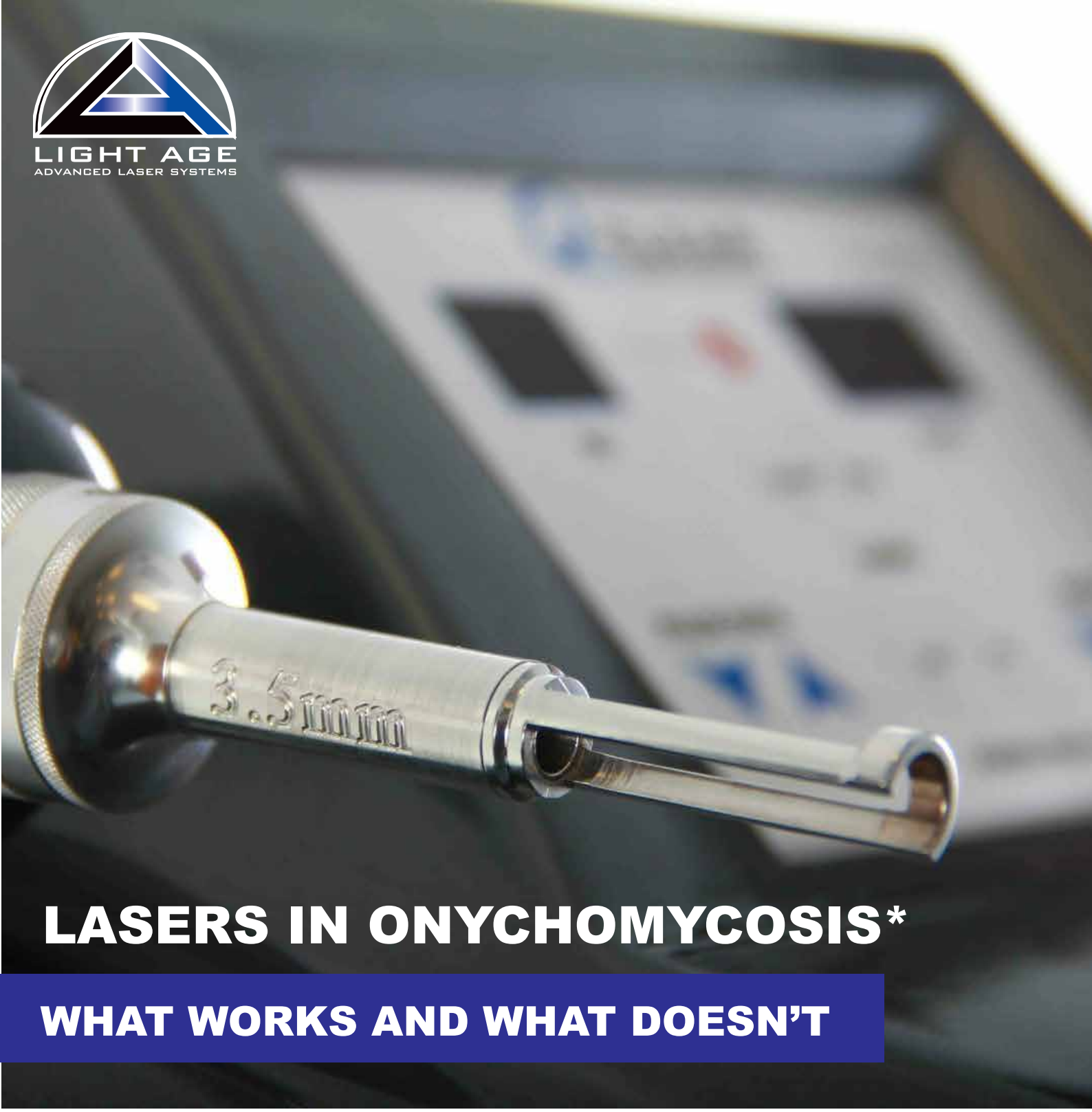

LASERS IN ONYCHOMYCOSIS*

WHAT WORKS AND WHAT DOESN'T

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WHAT WORKS

WHAT DOESN'T WORK

LIGHT AGE
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WORKS

The Effect of Q-Switched Nd:YAG 1064 nm/532nm Laser in the Treatment of Onychomycosis In Vivo

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Clinical Study

The Effect of Q-Switched Nd:YAG 1064 nm/532 nm Laser in the Treatment of Onychomycosis In Vivo

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In this prospective clinical study, the Q-Switched Nd:YAG 1064 nm/532 nm laser (Light Age, Inc., Somerset, NJ, USA) was used on 131 onychomycosis subjects (94 females, 37 males; ages 18 to 68 years). Mycotic cultures were taken and fungus types were detected. The laser protocol included two sessions with a one-month interval. Treatment duration was approximately 15 minutes per session and patients were observed over a 3-month time period. Laser fluencies of 14 J/cm^2 were applied at 9 billionths of a second pulse duration and at 5 Hz frequency. Follow-up was performed at 3 months with mycological cultures. Before and after digital photographs were taken. Adverse effects were recorded and all participants completed "self-evaluation questionnaires" rating their level of satisfaction. All subjects were well satisfied with the treatments, there were no noticeable side effects, and no significant differences were found treating men versus women. At the 3-month follow-up 95.42% of the patients were laboratory mycologically cured of fungal infection. This clinical study demonstrates that fungal nail infections can be effectively and safely treated with Q-Switched Nd:YAG 1064 nm/532 nm laser. It can also be combined with systemic oral antifungals providing more limited treatment time.

1. Introduction

Onychomycosis is defined as a fungal infection of the nail that expands slowly and if left untreated leads to complete destruction of the nail plate. Onychomycosis can be dermatophytic (99%) and/or nondermatophytic (1%) (including yeasts) infections of the nail plate.

The dermatophytes *Trichophyton rubrum* and *Trichophyton mentagrophytes* are the most common causative pathogens responsible for up to 90% of all cases [1]. Onychomycosis represents about 30% of all dermatophyte infections and accounts for 18%–40% of all nail disorders. The prevalence of onychomycosis ranges between 2% and 28% of the general population and it is estimated to be significantly higher in specific populations such as in diabetes mellitus, the immunosuppressed, and elderly [2, 3].

Among the nondermatophytes, the yeast *Candida albicans*, *Candida tropicalis*, *aspergillus*, and other molds may be responsible. It usually represents contamination and is an emerging problem in HIV patients.

Toenails are far more likely to be involved than fingernails. Initially solitary nails are involved; later, many may be infected, but often one or more can stay disease-free. Onychomycosis has no tendency for spontaneous remission and

should be considered as a problem with serious medical, social, and emotional extensions, not solely a cosmetic problem. The primary concerns of the patients are the risk of spread to other nails or to people in their environment. Others consider their deformed nails as unattractive to other people, which may lead to lower self-esteem, a sense of inadequacy, and even depression [4, 5]. In addition to these social and emotional problems, onychomycosis is a serious medical problem that can be the source of further fungal infections to surrounding tissues. Also, it may predispose patients to secondary bacterial infections leading to localized paronychia and perhaps worse and deeper infections such as erysipelas-cellulitis, especially in the high-risk groups such as diabetics [6, 7]. Clinically it can cause varying degrees of pain or discomfort (especially in walking) and problems in cutting nails.

Classical treatment options include mechanical and chemical debridement, topical antifungal lacquers, systemic antifungal drugs, and finally various combinations of the above. The most effective mono-therapies for onychomycosis are antifungal agents which have been the gold standard and mainstay of therapy for years. The downside of the antifungals are that they require blood testing to monitor the liver because they are systemic and also that they require long treatment courses (approximately 6 months for toenails and 4 months for fingernails). This requires liver function-transaminases and kidney function blot test control. Patients may also receive concomitant medications for comorbidities, so there is also the issue of drug interactions. Additionally, long lasting treatment means high treatment costs for both the patient and health insurers. Finally, high recurrence rates have been described, 22% three years after completion of treatment and higher recurrence rates at five years follow-up [8–10].

Recently, lasers have emerged as potential new treatment modalities. These treatments offer the advantage of having few contraindications and minimal side effects [11–13]. Laser energy has the potential to eliminate microorganisms. Vural et al. recently demonstrated direct inhibitory activity of laser energy on *T. rubrum* isolates in vitro [14]. Manevitch et al. recently published the direct antifungal effect of the femtosecond laser on *T. rubrum* onychomycosis as well [7]. The laser must have the ability to penetrate under the nail plate in order to reach the fungi colonies of the nail bed and nail matrix. When it gets to that point it should selectively deliver laser energy to fungi while respecting the surrounding healthy tissues.

In this study we planned to evaluate the effect of the neodymium: yttrium-aluminum-garnet (Nd:YAG) 1064 nm/532 nm laser in the treatment of onychomycosis in vivo.

2. Material and Methods

2.1. Nail Sampling and Fungal Cultures. Nail cuttings sized 2 × 3 mm were obtained from patients with clinical suspicion of onychomycosis. After direct microscopy to observe spores, hyphae, mycelia, and colonies of the latter, samples were plated on *Sabouraud glucose agars* with *cyclohexamide* to select for dermatophytes, in order to verify fungal infection. Cultures were incubated at 28°C for 3 weeks until fungal colonies developed.

2.2. Evaluation of Fungal Elimination. Before the treatment culture was performed and 4 weeks after the second treatment session (8 weeks after the first positive culture), culture was repeated. Mycological cure is defined as negative microscopy and culture. Clinical cure is associated with the alteration of the percentages of disease-free nails. Complete cure is accepted as the combination of mycological and clinical cure. Three months after the first treatment session, laser treatment was evaluated [15, 16].

2.3. Inclusion Criteria. To take part in the study each patient had to have one or more toenail and/or fingernail fungal infections of the following types: distal subungual onychomycosis, proximal subungual onychomycosis, superficial white onychomycosis, or total dystrophic type onychomycosis. Patients with diabetes mellitus, immunocompromised patients, and organ transplant patients were also included, although we considered that these patient groups success rates could be considerably less.

2.4. Exclusion Criteria. Patients who used systemic antifungal or isoretinoin within 6 months of the first scheduled laser session were excluded. The following conditions, which can cause various physiological changes to the nail plate, were also excluded: subungual hematoma, nevoid subungual formation, bacterial nail infections, concomitant nail disorders due to psoriasis, atopic dermatitis, lichen planus, and pregnant women were not included.

2.5. Pretreatment. As onychomycosis causes significant thickening (hyperkeratosis) of the nail plate, before starting our laser sessions we performed the mechanical debridement of any excessive nail thickness. This procedure was conducted with a file by a trained podiatrist. This mechanical debridement alone does not constitute an effective treatment, but it helps the laser penetrate under the nail plate to reach the fungal colonies of the nail bed and nail matrix.

2.6. Grading the Severity of Onychomycosis: Onychomycosis Severity Index. The Onychomycosis Severity Index (OSI) score is obtained by multiplying the score for the area of involvement with a range of 0–5 (1–10% is rated with 1, 11–25% with 2, 26–50% with 3, 51–75% with 4, and finally 76–100% with 5) by the score for the proximity of disease to the matrix with also a range of 1–5. Ten points are added for the presence of a longitudinal streak or a patch (dermatophytoma) or for greater than 2 mm of subungual hyperkeratosis. Mild onychomycosis corresponds to a score of 1 through 5; moderate, 6 through 15; and severe, 16 through 35. All patients were examined monthly for the evidence of proximal extension of the nail bed lesion. Any proximal extension of the lesion during treatment was a treatment failure [17, 18].

2.7. Laser Irradiation. The irradiation was performed with a Q-Switched Nd:YAG 1064 nm (Q-Clear, Light Age, Somerset, New Jersey, USA). Laser protocol was performed with 2.5 mm spot size and a power level of 4 which delivers 14 joules/cm^2, 9 billionths of a second pulse duration, and a 5 Hz frequency.

The second pass was done with the same laser operating at 532 nm Nd:YAG with the following parameters: 2.5 mm spot size and a power level of 4 which delivers 14 joules/cm^2, 9 billionths of a second pulse duration, and a 5 Hz frequency. No local anesthesia was applied preoperatively.

In one session two passes across each nail plate were performed with two minutes pauses between each pass. The first pass was performed with the 1064 nm Nd:YAG laser. Each nail was fully covered with a laser beam, including the areas of the hyponychium and the proximal and lateral nail folds. After a two minute intermission the second pass was performed with the 532 nm Nd:YAG, fully covering the nail plate but not the hyponychium and nail folds. All patients were also evaluated with posttreatment fungal cultures.

Postoperative analgesic treatment was not required. No prophylactic antibiotics or antivirals were given to any patient.

The full treatment consisted of two sessions executed on days 0 and 30. Nails were photographed with a high-resolution digital camera before treatment at day 0 (pre-treatment photograph). Follow-up visits were done at day 30 (before the second session). Photographs were taken again using the same camera settings, with lighting and nail position at baseline and day 60.

3. Results

3.1. Clinical Onychomycosis Types. Patients had all four major clinical types of onychomycosis: distal subungual onychomycosis, proximal subungual onychomycosis, superficial white onychomycosis, or dystrophic type onychomycosis. Another group is onychomycosis that affects only the lateral edge. The clinical onychomycosis types separated by gender and age group are given in Table 1.

Distal subungual is the most common clinical type of onychomycosis among both genders and all age groups since it appears in 123 (93.9%) of the total patients, followed by lateral edge (in 47 patients (35.9%)), dystrophic type (in 13 or 9.9%), superficial white (in 2 patients or 1.5%), and, last, proximal subungual (in only 1 patient or 0.8%). Moreover, 94.7% of female patients, 91.9% of the males, 95% of patients under 30 years old, 93% between 30 and 60 years old, and 95.8% over 60 suffer from distal subungual. The corresponding counts and percents for the rest of the clinical types of onychomycosis may be seen in Table 1.

3.2. Fungus Types. The most frequent fungus found among treated patients was *T. rubrum* (in 108 patients or 82.3%), followed by *Candida* (in 19 patients 14.6%) and then *Trichophyton mentagrophytes* (in 4 patients 3.1%). Table 2 presents the types of fungi found in patient populations and their percentages. The fungus types can also be seen by patient's ages and genders.

3.3. Severity of Onychomycosis. Table 3 shows all patients according to onychomycosis severity.

Regarding the severity of onychomycosis, severe onychomycosis seems to be more frequent in men (78.4% versus 62.8%). A chi-square test for the differences between genders suggested that those differences are not statistically significant at any significance level ($\chi^2 = 3.681$, $P = 0.159$). We draw the same conclusions from a chi-square test for the differences in age ($\chi^2 = 3.002$, $P = 0.557$).

We also evaluated our patients according to great nail and/or multiple nail involvement (Table 4).

3.4. Mycologic Cure of Nail Fungal Infections. At 3-month follow-up 125 patients (95.42%) showed mycological cure (negative microscopy and culture). There was no treatment failure (proximal extension of the lesion during treatment). Clinical cure is associated with the alteration of percentages of disease-free nail. We find a change of >76% as excellent response, 51–75% as very good response, 26–50 as good response, 6–25% as moderate response, and >5% as low response to treatment.

It can be seen in Table 5 that the clinical type of onychomycosis seems to have an important influence on response: "distal subungual" had the best response followed by "lateral edge, dystrophic type, and superficial white"; however "proximal subungual" type showed the lowest response.

Dermatophytes (*T. rubrum*) seem to have the best response rate followed by *Trichophyton mentagrophytes* and *Candida* comes last. Paradoxically, *moderate* onychomycosis showed the best results, while *mild* is next and *severe* last.

The age group under 30 revealed the best results, additionally women showed the best response (Figures 1(a), 1(b), 2(a), 2(b), 3(a), 3(b), 4(a), 4(b), 5(a), 5(b), 6(a), 6(b), 7(a), and 7(b)).

Among the above differences, only three are statistically significant.

The following are the differences.

(i) Genders: women seem to be cured more effectively than men do at a 5% significance level ($f = 5.237$ and P-value = 0.024).

(ii) Severity of onychomycosis: mild severity patients are cured most effectively, followed by moderate severity and lastly severe severity patients at a 1% significance level ($f = 9.963$ and P-value = 0.00).

(iii) The responsible nail fungi: *T. rubrum* recedes more quickly after the cure, followed by *trichophyton mentagrophytes* and *Candida* at a 1% significance level ($f = 15.347$ and P-value = 0.00).

3.5. Adverse Event Evaluation. Most patients, 94 (83.21%), reported mild pain; 22 patients (16.79%) reported no pain. This "pain" sensation was described as "a stinging" during the 1064 nm pass and as "burning" during the 532 nm pass. None of the patients treated had severe or intolerable pain. No postoperative analgesic treatment was required. Interestingly many of patients developed a kind of pain resistance during the therapy, meaning they reported the highest level of pain

Table 1: Clinical onychomycosis types.

Patients	Total	Distal subungual	Proximal subungual	Superficial white	Dystrophic type	Lateral edge
	131 (100.0%)	123 (93.9%)	1 (0.8%)	2 (1.5%)	13 (9.9%)	47 (35.9%)
Gender						
Female	94 (71.8%)	89 (94.7%)	0 (0.0%)	2 (2.1%)	9 (9.6%)	26 (27.7%)
Male	37 (28.2%)	34 (91.9%)	1 (2.7%)	0 (0.0%)	4 (10.8%)	24 (56.8%)
Age group						
<30	20 (15.3%)	19 (95.0%)	0 (0.0%)	1 (5.0%)	4 (20.0%)	1 (5.0%)
30–60	86 (65.6%)	80 (93.0%)	0 (0.0%)	1 (1.2%)	7 (8.1%)	31 (36.0%)
>60	25 (18.3%)	23 (95.8%)	1 (2.7%)	0 (0.0%)	2 (8.3%)	14 (58.3%)

Table 2: Fungal culture results and distribution according to age and gender.

Patients	Fungus type			
	Trichophyton rubrum	*Candida*	*Trichophyton mentagrophytes*	All types of onychomycosis
	108 (82.3%)	19 (14.6%)	4 (3.1%)	131 (100.0%)
Gender				
Female	79 (84.0%)	12 (12.8%)	3 (3.2%)	94 (100.0%)
Male	29 (78.4%)	7 (18.9%)	1 (2.7%)	37 (100.0%)
Age Group				
<30	19 (95.0%)	1 (5.0%)	0 (0.0%)	20 (100.0%)
30–60	71 (82.6%)	12 (14.0%)	3 (3.5%)	86 (100.0%)
>60	17 (70.8%)	6 (25%)	1 (4.2%)	24 (100.0%)

Table 3: Onychomycosis severity index [15, 17] with age and gender relation.

Patients	Mild (1–5)	Moderate (6–15)	Severe (16–30)
	6 (4.6%)	37 (28.2%)	88 (67.2%)
Gender			
Female	4 (4.3%)	31 (33.0%)	59 (62.8%)
Male	2 (5.4%)	6 (16.2%)	29 (78.4%)
Age group			
<30	1 (5.0%)	5 (25.0%)	14 (70.0%)
30–60	5 (5.8%)	27 (31.4%)	54 (62.8%)
>60	0 (0.0%)	5 (20.8%)	19 (79.2%)

Table 4: Evaluation of patients to multiple nail involvements.

Patients	Great nail involvement	Multiple nail involvement
	74 (56.5%)	33 (25.2%)
Gender		
Female	58 (61.7%)	27 (28.7%)
Male	16 (43.2%)	6 (16.2%)
Age group		
<30 y.o	7 (35%)	5 (25.0%)
30–60	54 (62.8%)	23 (26.7%)
>60	12 (50%)	5 (20.8%)

during the first session. We believe this suggests the patients knew what to expect or that the fear of an unknown treatment no longer existed.

Patients were also asked to report all possible adverse events that could be related to our treatment. There were no reports of any other side effects.

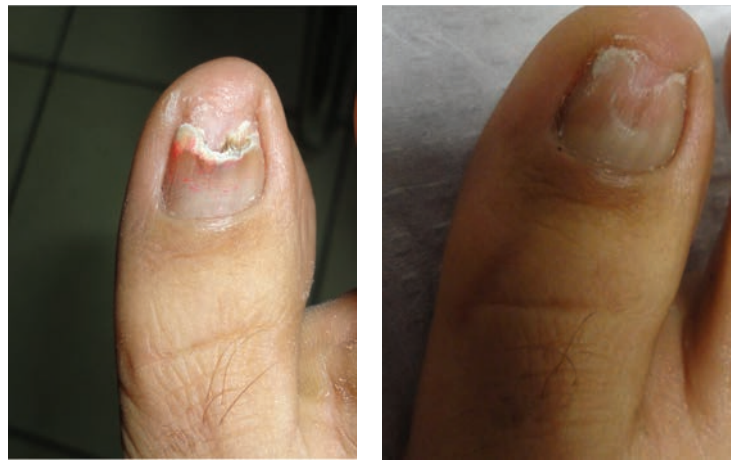
4. Discussion

Treatment of onychomycosis is difficult. Laser treatment is considered by some authors to be a promising new method. Our study population comprised of 131 individuals. 15.3% of the participants in the study were below 30 years of age, 65.6% between 30 and 60 years, and finally 18.3%, were over 60 years old. These groups allowed us to maintain a large enough sample within each group to compare the effectiveness of the laser treatment on different age groups. Women were the 71.8% of our patient sample. This does not mean that onychomycosis occurs more frequently in women but that men may be more negligent in matters relating to the cosmetic appearance and hygiene of their feet.

In a recent paper Vural et al. showed that 1064 nm and 532 nm Q-Switched Nd:YAG laser systems had significant inhibitory effect upon *T. rubrum* isolates and caused colony growth inhibition in vitro [14]. It is well known that the efficacy of laser energy depends on the light-tissue interaction which is a function of wavelength, fluence, and tissue optics [7]. We have used various spot sizes in all power levels with our system. This can provide combinations which deliver different energy fluence. We found that the most powerful treatment was 14 joules/cm^2; additionally, the 7.5 joules/cm^2 (3.5 mm spot size and a power level of 4) was also effective. Since the treatment session is very well tolerated in the maximum energy fluence, we used these settings. We have noticed a significant improvement in the proximal

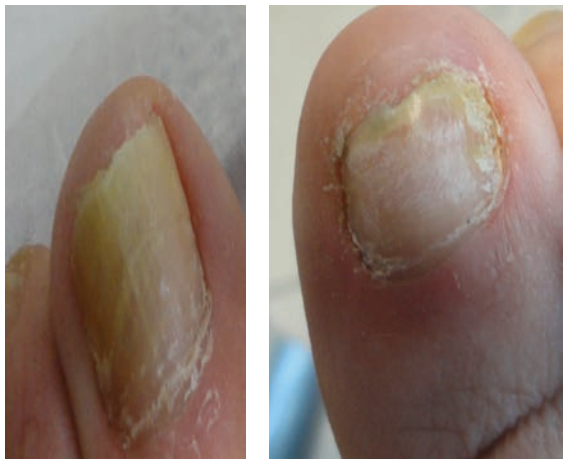
Table 5: Laser treatment response according to age, gender, type of fungi, clinical type of onychomycosis, and location.

Patients	Excellent response (>75%)	Very good response (50–74)	Good response (25–49)	Moderate response (10–24%)	Low response (>9%)	No response (0%)
Gender						
Female	10 (10.6%)	44 (46.8%)	25 (26.6%)	10 (10.6%)	0 (0.0%)	5 (5.3%)
Male	2 (5.4%)	9 (24.3%)	16 (43.2%)	9 (24.3%)	0 (0.0%)	1 (2.7%)
Age						
<30 y.o	3 (15.0%)	4 (20.0%)	10 (50.0%)	2 (10.0%)	0 (0.0%)	1 (5.0%)
30–60	9 (10.5%)	37 (43.0%)	24 (27.9%)	13 (15.1%)	0 (0.0%)	3 (3.5%)
>60	0 (0.0%)	12 (50.0%)	6 (25.0%)	4 (16.7%)	0 (0.0%)	2 (8.3%)
Onychomycosis severity						
Mild	3 (50.0%)	2 (33.3%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Moderate	5 (13.5%)	23 (62.2%)	9 (24.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Severe	4 (4.5%)	28 (31.8%)	31 (35.2%)	19 (21.6%)	0 (0.0%)	6 (6.8%)
Types of fungi						
T. rubrum	10 (9.3%)	51 (47.2%)	38 (35.2%)	8 (7.4%)	0 (0.0%)	1 (0.9%)
Candida	1 (5.3%)	1 (5.3%)	3 (15.8%)	10 (52.6%)	0 (0.0%)	4 (21.1%)
Non dermatophytes	1 (25.0%)	1 (25.0%)	0 (0.0%)	1	0 (0.0%)	1 (25.0%)
T. mentographytes	9 (9.4%)	48 (50.0%)	35 (36.5%)	4 (4.2%)	0 (0.0%)	0 (0.0%)
Clinical type of onychomycosis						
Distal subungual	9 (7.3%)	50 (40.7%)	40 (32.5%)	18 (14.6%)	0 (0.0%)	6 (4.9%)
Proximal subungual	0 (0.0%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Superficial white	1 (50.0%)	0 (0.0%)	0 (0.0%)	1 (50.0%)	0 (0.0%)	0 (0.0%)
Dystrophic	2 (4.3%)	14 (29.8%)	17 (36.2%)	11 (23.4%)	0 (0.0%)	3 (6.4%)
Lateral edge	2 (4.3%)	5 (38.5%)	5 (38.5%)	0 (0.0%)	0 (0.0%)	1 (7.7%)
Location						
Hand	0 (0.0%)	1 (9.1%)	3 (27.3%)	6 (54.5%)	0 (0.0%)	1 (9.1%)
Feet	9 (9.9%)	38 (41.8%)	27 (29.7%)	14 (15.4%)	0 (0.0%)	3 (3.3%)



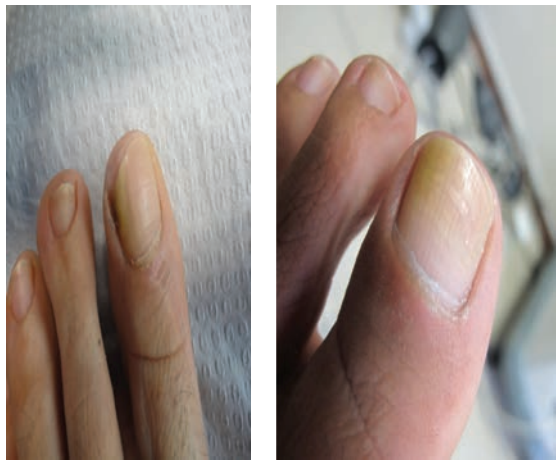
(a) (b)

Figure 1: (a) 68-year-old female patient, before treatment. *Trichophyton rubrum* was isolated on mycological testing. Onychomycosis severity index (OSI) was 16. (b) After treatment with good improvement. OSI is 6 showing 69.5% improvement.



(a) (b)

Figure 2: (a) 64-year-old female patient before treatment. *Trichophyton rubrum* was isolated on mycological testing. OSI is 26. (b) After treatment with great improvement. OSI is 9 showing 65.38% improvement.

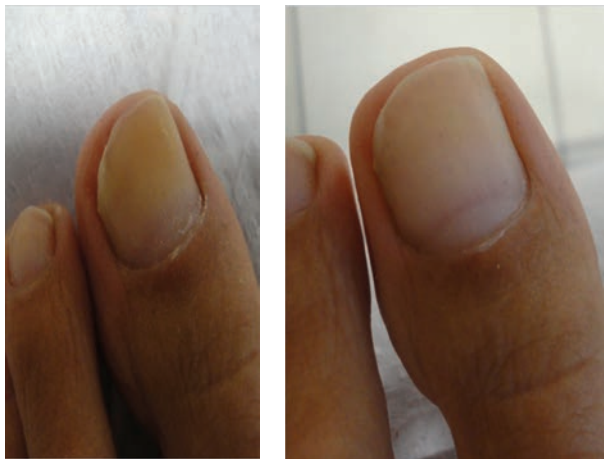


(a) (b)

Figure 3: (a) 48-year-old female patient before treatment. *Trichophyton rubrum* was isolated on mycological testing. OSI was 9. (b) After treatment with great improvement. OSI is 2, showing 77.78% improvement.

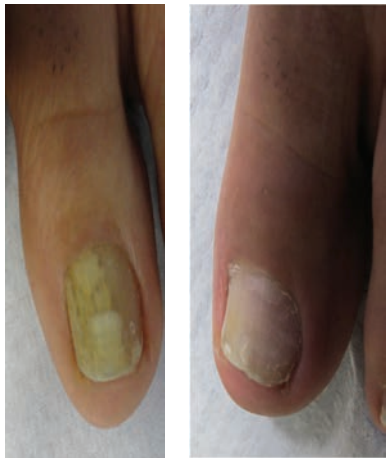
portion of the nail where there was mild initial mycotic involvement. Our results were better especially in moderate severity patients. That seems reasonable as severe cases are accompanied by dermatophytoma or significant subungual hyperkeratosis, which require more time for the nail plate to restore. Poor prognostic indicators are the total dystrophic onychomycosis, the involvement of the lateral edge of the nail plate, and the involvement of the matrix [18–21]. The thick plate or subungual hyperkeratosis >2 mm histologically contains numerous air-filled spaces in which fungal spores can survive for weeks or months. These resting arthrospores do not form hyphae, so various antifungal agents have proven ineffective. This phenomenon, termed as dermatophytoma, can be seen as linear streaks or rounded white areas in the nail plate. The fungal elements are believed to be forming a biofilm, making them refractory to therapy [15, 22, 23]. Laser therapy seems not to be affected of this biofilm formation; this may explain why we achieved very good and good response in 67% of our severe cases. Moreover, old age, presence of immunosuppression, poor peripheral circulation and nonresponsive organisms (nondermatophyte molds), other dermatoses (e.g., nail psoriasis), and drug resistance are poor prognostic indicators [15, 23, 24]. With the laser we solve the problem of resistance. We suggest that we do not have nonresponsive cases but some poor responding fungi. As another example, occupational factors, as well as occlusive and prolonged contact with water, can contribute to poor response of treatment [21].

On the contrary, superficial white onychomycosis is associated with the best therapeutic response to antifungal drugs, and our results seem to agree with this [16, 19]. Our distal subungual clinical cases had good results as well. Even dystrophic types showed a very good and good response in 66% of the cases. This supports laser treatment efficacy. Laser treatment



(a) (b)

Figure 4: (a) 28-year-old female patient before treatment. *Trichophyton rubrum* was isolated on mycological testing. OSI was 12. (b) After treatment with great improvement. OSI is 1, showing 91.67% improvement.



(a) (b)

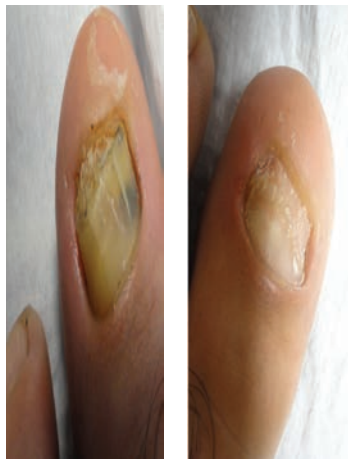
Figure 5: (a) 60-year-old female patient, before treatment. *Verticillium sp.* was isolated on mycological testing. OSI was 30. (b) After treatment with great improvement. OSI is 4 showing 86.67% improvement.

seems to outweigh classical treatments where involvement of the matrix, a thick plate, or subungual hyperkeratosis >2 mm are factors associated with poor outcome [15, 21].

The Q-Clear Laser System, in differentiation to other laser treatments, provides a selective, both thermal (photothermolytic) and mechanical (photomechanical), effect on the fungi. The mechanism of this fungal destruction may offer some differences. The inhibitory effect is likely due to more than simple nonspecific thermal damage. Denaturing one or more of the molecules within the pathogen may deactivate the fungi. Vural et al. discusses that 532 nm setting, which is well absorbed by red pigment in canthomegnin in *T. rubrum*, this wavelength generates mechanical damage in the irradiated fungal colony [14].

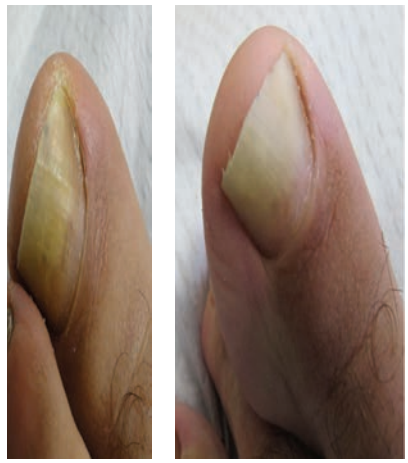
The 1064 nm setting is beyond the absorption spectrum of xanthomegnin, but its effectiveness is due to another absorbing chromophore, perhaps melanin, which is present in the fungal cell wall [14]. Melanin is an essential inhabitant of the fungal cell wall and has been described in many pathogenic species. The type of melanin varies, although it is commonly Dopa or pentaketide melanin. Moreover, the laser beam may initiate a photobiological or photochemical reaction that attacks the pathogen cell. We can also suggest a multiphoton dielectric breakdown at the fungal target as the cause of their destruction, while depth-selective thermal effects by the laser could also be occurring [7].

Another possible scenario is by inducing an immune response that attacks the organism. All of the above hypotheses explain how the surrounding host tissue cells are protected from this attack, with little or no collateral damage. The amount of energy delivered by our treatment session may serve as a deactivating dose. That amount of energy can



(a) (b)

Figure 6: (a) 32-year-old male patient, before treatment. *Trichophyton rubrum* was isolated on mycological testing. OSI was 35. (b) After treatment with great improvement. OSI is 12 showing 65.71% improvement.



(a) (b)

Figure 7: (a) 58-year-old male patient, before treatment. *Trichophyton rubrum* was isolated on mycological testing. OSI was 30. (b) After treatment with great improvement. OSI is 9 showing 70.00% improvement.

deactivate 80–99% of the organisms present in an affected nail without instantly killing the fungal colonies but it can disable their ability to replicate or survive according to an apoptotic mechanism. Apoptosis, a physiological type of cell death, plays an important role in the selective deletion of cells in divergent situations of various tissues [25]. Induced apoptosis may cause direct DNA damage, for example, strand breaks, chromosomal aberrations, induction by transduced signals, for example, FAS/APO-1 transmembrane signals, and stress (heat) mediated death. Hyperthermia, a typical environmental stress, has long been known as toxic to cells. It has been recognized the mode of cell killing to be influenced by the severity of the heat treatment [26]. A number of reports have been published to demonstrate the induction of apoptosis by mild hyperthermia [27, 28].

We are waiting to assess our results following twelve months since the completion of treatment, which is the time required for complete regeneration of the nail plate. Additionally, we will follow the patients at greater time intervals to assess the occurrence of relapse. Zaias et al. recommended that the treatment of onychomycosis with oral antifungals should be continued until the mycotic nail bed had been completely replaced by a new mycotic bed (that requires about 12 months for toenails). With this treatment the authors achieved significantly better cure rates [18]. It may be that this maintenance therapy will provide a safety net for those at risk of relapse after the discontinuation of laser treatments.

In contrast to our results, recently Carney et al. evaluated thermal response and optical effects of a submillisecond neodymium: yttrium-aluminum-garnet (Nd:YAG) 1064 nm laser on common fungal nail pathogens and the clinical efficacy and safety laser of onychomycotic toenails. A fungicidal effect for *T. rubrum* was seen at 50°C after 15 minutes and for Epidermophyton floccosum at 50°C after 10 minutes. No inhibition was observed after laser treatment of fungal

colonies or suspensions. In vivo treatment of toenails showed no improvement in Onychomycosis Severity Index score. They discussed that laser treatment of onychomycosis was not related to thermal damage or direct laser effects [29].

Similarly Hees et al. were also unable to show the effect of Nd:YAG laser on *T. rubrum* colonies. They assumed that the effect could be due to unspecific tissue heating with a subsequent increase in circulation and stimulation of immunologic process. They also discussed the associated risks of laser treatment with the use of higher densities [30]. Laser systems vary widely and it is understandable that there are differeing results. The Q-Clear's 1064/532 nm wavelengths and unique time-structured pulse profile specifically target the fungal elements, inducing a progressive and eventually lethal temperature increase. At the same time the low-absorption, high water content tissues (dermal), and vascular flow, allow rapid dissipation of absorbed energy, thus "antitargeting" the nail bed and other dermal tissues.

Competing "long pulse" systems presumably relay on bulk heating of fungal colonies in situ on the nail bed with the associated discomfort which necessitate multiple treatments and a high treatment failure rate. Some of the papers in the literature calling laser a failure were also only Petri dish studies which cannot replicate these in vitro applications.

Although some studies have yielded conflicting results, other studies like ours have shown some promise [31–34].

Zhang et al. had satisfactory results with the Nd:YAG without significant complications. They discussed that the thicker the nail plates the higher the laser energy needed to be. Different fungal strains may also have different sensitivities [32]. Hochman [33] and Bornstein et al. [34] described the formation of free radicals as well as the influence of the laser on cellular reaction. These results support our study.

Finally, we find the treatment of onychomycosis with this specific Q-Switched Nd:YAG, 1064 nm/532 nm laser in vivo as extremely promising and efficient. In addition, laser-based treatments have the advantage of a regimen that is devoid of mutagenic and genotoxic effects. They could be combined with systemic oral antifungals providing the benefit of limiting treatment time.

4.1. Weaknesses of the Research. Whereas the present study demonstrates the efficacy of the specific laser in the treatment of onychomycosis, we should keep in mind that negative cultures, that is, mycological cure, do not always constitute proof of clinical cure due to the well-known high rate of false-negative culture results.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

Kostas Kalokasidis, Myrto-Georgia Trakatelli, and Bertrand Richert performed this clinical trial. Meltem Onder and Klaus Fritz did scientific evaluation.

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LIGHT AGE
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WORKS

A Clinical Study of Q-Clear™ Laser-Treatment of Distrophic Nails. (US FDA Submission 2011)

4 - 44

A Clinical Study of Q-Clear™ Laser-Treatment of Dystrophic Toenails

A Single Site*, Multiple-Practitioner, Blind-Coded Study of 100 Cases with Clinically Apparent Diagnosis of Onychomychosis

Submitted by

Light Age, Inc. in support of its pending application for market clearance for additional indication in the clearance of dystrophic nails.

*Study Conducted at Southwest Foot and Ankle Associates, Inc., L. Jon Schurmeirer Medical Pavilion, Southwest General Health Center, 7255 Old Oak Boulevard, Suite C-308 Middleburg Heights, Ohio 44130

Principal Investigator: Dr. James R. Holfinger, DPM

LIGHT AGE INCORPORATED

Purpose:

The purpose of the study was to investigate, quantify, and correlate nail plate clearance subsequent to laser treatment of a population of non-specifically diagnosed dystrophic toenails. Although it was not the purpose of this clinical study to associate observed nail dystrophy with any specific cause or disease, and no mycology was performed, all subjects had initial, clinically apparent diagnosis of onychomychosis. Most study subjects reported a protracted history of toenail dystrophy and had been highly motivated in seeking various treatments including both topical and systemic medications.

While there are many causes for toenail dystrophy, including various infectious agents, irritants, trauma, and congenital diseases and susceptibilities, studies indicate that onychomychosis is a major cause, reported in some studies to occur in over 13% of the population and accounting, by some estimates, for up to 50% of all nail diseases. (Scher, R. K and Daniel, C. R., 2005; emedicine-online, 2011) and up to 90% in elderly patients. This said, it is noted that today some controversy exists as to the dominant infectious entities responsible for onychomychosis, although there is a fair consensus on T.rubrum and similar dermatophytes, however, both causative agent and morbidity apparently differ from region to region throughout the world. Generally indicated mycotic agents include: T. rubrum and other dermatophytes, Candida albicans, and various non-dermatophyte molds and yeasts (*ibid*.). Additionally, factors contributing to the increasing morbidity includes increasing incidence of lower extremity circulatory disease, auto-immune diseases, climatic change, and aging populations. While there is a large and growing literature on the subject, the present clinical study was disinterested in addressing anything but the specific issue of laser-induced clearance of dystrophic toenails.

Composition of the Study:

A subject group of 100 hallux toenails from 85 patients were randomly selected from a larger population of approximately 400 dystrophic toenails treated with a Q-Clear™ Nd:YAG laser system. All subjects were treated at a single clinical site (Southwest Foot and Ankle Associates, Inc., Cleveland, OH) beginning in March 2010. When treatments began, all subject toenails were laser treated together with all other patient toenails, whether or not any of the other toenails were dystrophic. Later, as the study progressed, only symptomatic nails were treated. Early treatments, for a very brief period of time, also included use of topical anti-fungal agents, although this practice was later halted. Early studies employed a range of treatment fluences, but this range was restricted as the study progressed. In all cases before and after treatment photographs were taken. To reduce bias, no contemporaneous assessment or quantification of nail clearance was performed until later in the study.

Hallux nails were selected for two reasons: 1. They are the most commonly affected nails and 2. The larger area of the nail plate facilitates quantification of laser induced clearance. It is noted that toenail infections are far more prevalent than fingernail infections in virtually all regions of the world.

The subject sample was down-selected from a larger patient population, based only on the apparent cause of the dystrophy not being ascribed to specific trauma or specific disease. Excluded were patients actively taking anti-fungal medications [e.g., Lamisil (terbinafine)].

Subjects ranged in age from 18 to 88. Gender composition was approximately male 28% and female 72%. All subjects were required to be above the age of consent (18 in OH). No selection or record of ethnicity was made, although the statistical group was heavily dominated by patients having Fitzpatrick skin types I – IV. Since the laser system being employed was approved for treating skin types I-VI and 1064 nm light is not strongly absorbed by melanin, it is not viewed that this bias in the randomly down-selected population, while also reflected in the overall patient population of the clinic, would have any significant affect on the results or conclusions of this study – particularly regarding either safety or efficacy. That said, it is noted that the effects on the darker skinned sub-population, was found to be within the significance levels of the sample population as a whole.

Procedure:

Generally, the treatment protocol used in this study followed the treatment guidelines described in Chapter 9 of the revised Q-Clear™ manual co-submitted with this application.

Post-Selection Interview:
Subjects were initially questioned regarding their history with dystrophic nails and prior and present treatment regimes. All were counseled to take post-treatment precautions to mitigate the potential for reinfection; e.g., foot and nail hygiene, dry shoes and socks, frequent changes for both. No claims were made as to the expected benefits of treatment, only that the subject were expected to return at 3 month intervals over the next year for observation and possible follow-up treatment.

Pre-laser treatment:
In cases where there was substantial dystrophy, such that the nail plate was substantially discolored, excessively thickened or largely visually opaque, debridement of the plate was performed. In all cases photographs were taken before the first treatment and at each return visit, scheduled at nominal 30 day intervals.

Laser treatment:
The Light Age, Q-Clear™ (Q-switched, Nd:YAG) laser system was used exclusively throughout this study. 1064 nm wavelength was initially used exclusively, although a study is ongoing to make a preliminary assessment of the benefit of using the 532

nm wavelength (the laser's second harmonic) to treat refractory subjects. The laser fluence was set by selection of laser power level setting and selection of spot size using the appropriate standoff. The pulse repetition rate was selected for convenience of patient and clinician. As the study progressed higher pulse rates (up to 5 Hz) were used. The entire nail unit and immediately surrounding tissues were carefully scanned to insure that all areas of the nail, symptomatic or not, were treated. Plates were scanned both horizontally and vertically. Before and during treatment the patient was asked repeatedly if he/she experienced any discomfort or pain.

Post-laser treatment:
Initially, for a very short period of time, laser treatment was augmented with application of topical antifungal medications, but this protocol was quickly suspended; there appeared to be no direct benefit, and there was some concern of contaminating the study findings. While use of antifungals might be of some use in prevention of re-infection, assessing that was not an aim of this study.

Side effects: aside from very slight erythema and occasional hypopigmentation of the skin dorsal to the proximal portion of the underlying nail plate and matrix, no significant side effects were noted, nor was their any complaint of pain or discomfort during or following treatment. No post-treatment edema or incidence of blistering were noted or reported.

Quantification and Data Analysis

The area of clear nail plate was measured from the photographs taken prior to and during the treatment period. Approximately 1 year after the study began, coded pictures of subject nails were blind-evaluated and each quantified as to the relative area of clear nail plate - from 0% to 100% (full plate clearance). Clearance of the initially dystrophic nail area was quantified by the metric: (post-pre)/(1-pre), where "pre" and "post" refer to the relative area of clear nail plate before the initial laser treatment and at the last assessment period for each subject in the study. The treatment fluence, the period of time from the initial treatment, and the number of treatments were recorded for statistical analysis. Data were analyzed from their histograms and by other standard statistical methodologies. (See results section below). Statistical evaluation included, among other parametric estimates, determination of the mean relative areal clearance of the initially compromised nail plate, assessment of the 98% confidence levels for variation of this sampling parameter from the true population mean, and assessment of likelihood of type 1 and type 2 errors in sampling. Analysis of the clearance distributions were performed for each nominal assessment period; that is, for subjects evaluated at under 6 months, between 6 and 9 months, and after 9 month post initial laser treatment. Because the distributions were typically non-normally distributed additional sampling tests were performed to insure that certain assumptions underlying the statistical assessments remained valid.

Clearance distributions were analyzed for subjects assessed prior to 6 months post initial treatment, between 6 and 9 months, and after 9 months (9-12 months) post initial treatment, as well as for all subjects regardless of length of time post initial treatment. Similar evaluations were made to analyze clearance dependence on fluence level and on later treatments,

The results of this analysis provided a determination of clearance efficacy following laser treatment. This was 56 ± 7% for all subjects, and varied only slightly from this for those subjects not cleared at prior assessment intervals, but cleared at later intervals as the nail plate grew out. Perhaps the most surprising and clinically important result is that 95% of all subjects treated in this study (and, anecdotally, in the large population as well) experienced significant and quantifiable nail plate clearance. Moreover, no significant side-effects were observed or reported by patients, and 100% of the subject population expressed satisfaction with the treatment, even those having little or no quantifiable plate clearance during the study. Somewhat surprisingly, these studies did not quantify significant additional clearance due to increasing laser fluence (above 5-8 J/cm2) or due to further laser treatment after the first. Nonetheless, the measured clearance of initially dystrophic nail plates was substantial and significant.

Discussion

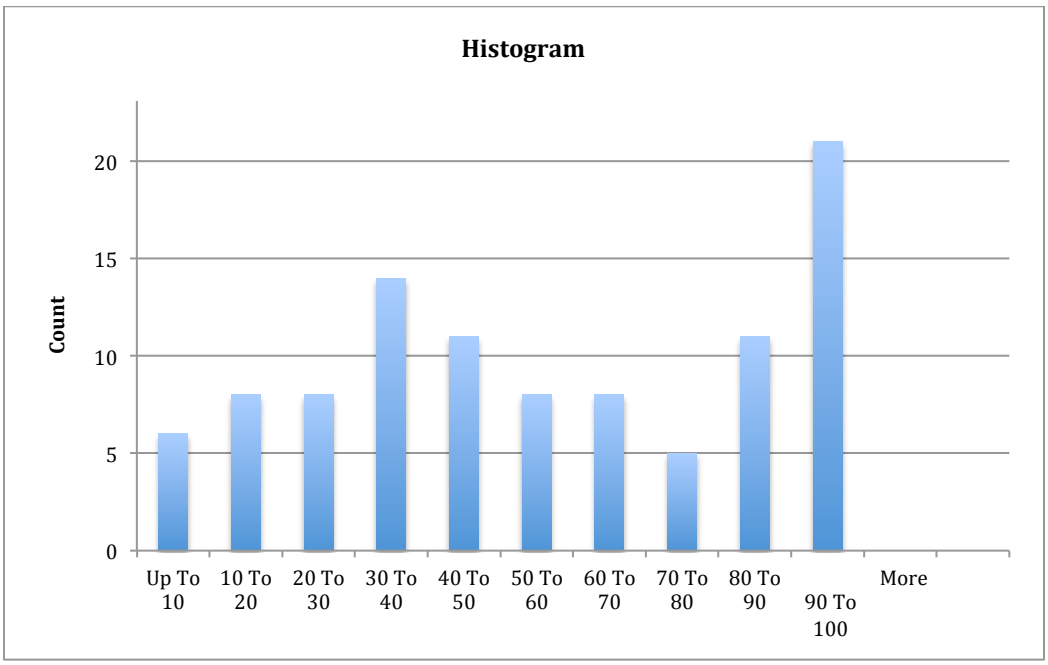
Analysis of Clinical Results

The results of this study, apparent in a survey of the before and after treatment photographs and quantified in our data analysis (above sections) have demonstrated a strong and highly significant clearance of dystrophic nails due to Q-Clear™ laser therapy when treatment is above 5 J/cm2 for the 1064 nm laser wavelength. Here we discuss our findings and certain of their implications and address the reasons why Q-Clear™ therapy is substantially more effective, and preferred, over treatments reported using long pulse lasers having more traditional temporal formats.

Our detailed results can be found in the accompanying appendices to this overview. Appendix A contains files of the initial and final photographs for each of the 100 subject cases in this study. These photographs were blind-scored and annotated for reference case number and patient initials, observation/treatment dates, laser treatment settings, photograph correspondence index, treatment comments, and initial/final scoring of clear nail area for each case. For conciseness, we have not included (either in the Appendix A photo files or in the summary tables in Appendix B) photographs taken at the intermediate sessions. That is, the initial photograph shows the subject toenail prior to any laser treatment and the “final” photograph shows the same toenail at the end of the present study period. The scored “Raw Data” is summarized in correspondingly labeled worksheet (Tab “1-Raw Data”) in the Appendix B Excel workbook. These data are quantified and recast in the next worksheet (Tab labeled “2-Data Quantification”. The next worksheet (Tab 3- All

Subjects) analyzes the study results for the entire subject sample, it summarizes the resulting nail clearance achieved during the study period independent of any other sample variables (fluence level, number of treatments, and elapsed time since first treatment). The following 4 worksheets break down the sample group into subgroups based on elapsed time since the initial laser treatment: Tabs#4.0–4.3 resolve the sample population into post-treatment periods (with respect to the initial Tx) of less than 6 months, 6 months to 9 months, and 9 months to 12 months, respectively). The final two worksheets (Tabs labeled: "5-Fluence Dependence" and "6-Treatment dependence") examine relationships between nail clearance and laser light dose (fluence) and between nail clearance and number of laser treatments, respectively.

The overview of treatment efficacy for the entire study group is given in Worksheet 3. The histogram there (and immediately below) shows that the distribution of clearance of nail dystrophy for the entire subject population at the end of the study period, independent of all other study variables. This shows that of the entire subject sample, consisting of 100 cases, at the end of the study period there was achieved an average of clearance of 56% of the initially affected nail plate area. Notable is the fact that this distribution is rather strongly bi-modal. This is expected from the observation that, as is typical of dystrophic nail populations where the predominant cause of dystrophy is from infection, generally initiating from proximal or distal edges of the nail plate, the disruption of the nail plate presents preponderantly near the proximal and distal edges of the plate. Due to this initially bi-modal spatial distribution of dystrophies, any early treatment that resolved or strongly mitigated the causative agent and permitted normal plate regrowth would result in more rapid full clearance from distally affected nails; proximately affected nails would take longer to grow out. This would naturally result in a bimodal distributional skewing when clearance was evaluated for the entire nail plate on timescales shorter than that needed for complete plate replacement for a substantial fraction of the sample population. Here 90% of the sample population that was evaluated at study end was less than 9 months post initial Tx. Statistical analysis revealed clearance levels, across the entire study group, averaging 56±7% of the initially dystrophic plate area at end of study. Analysis indicated that this result would be reproduced with 98% confidence. [Included in Worksheet 3 is test to verify that the statistical analysis was not meaningfully influenced by the fact that the sample distribution was not normally distributed.]



Histogram from Workbook, Tab-3 All Subjects: *Distribution of percentage clearance following Q-Clear™ laser treatment of initially dystrophic nail area (independent of position of dystrophy on the nail plate) for all study subjects at study end. At study end, subjects ranged from 2.8 to 12 months post entry (initial Tx). Note: Due to the metric used, all subjects entering the study (by definition) begin at 0% clearance.*

Probably our most notable finding, however, is the observation that 95% of subjects exhibited significant, quantifiable clearance of dystrophic nail plate areas during the study period (ranging from 2.8 to 12 months post initial treatment)! To our knowledge, this substantially exceeds the best results reported to date in the literature for any treatment protocol.

A further analysis of study data was undertaken to assess the potential for additional improvement of clearance results by a longer study period or with treatment fluence variations or with additional laser treatments. This analysis can be found in accompanying worksheets. Worksheets 4.0-4.3 examine the time evolution of clearance within the study population. This is influenced by plate grow out (see above) and by retreatment of a subset of the sample population. (The latter effect is examined more specifically in Worksheet #6.) As can be seen, by examining the histogram series in 4.1, 4.2, and 4.3 corresponding to sample subsets evaluated at periods of under 6, 6 to 9, and 9 to 12 months post initial treatment, respectively, there is a tendency for increasing clearance, particularly for the subjects showing least clearance at the earlier evaluation times. While this tendency prevails, there is little tendency for the mean clearance level to change over time. This may be influenced by the fact that the sample population includes nails with dystrophies due to a variety of causes, including those, such a trauma, which are not expected to be fully responsive to laser treatment. Although the clinically apparent predominant diagnosis for the study group was onychomycosis, studies reported in the literature

(and anecdotally) suggest that between 10 and 30% of such clinical diagnoses are not borne out by subsequent mycology. That said, far more likely is the related observation on the study population that some fraction of subjects, having suffered with symptoms for a protracted period (often many years) have dystrophies that have compromised the nail matrix and nail bed. As has been reported in multiple prior studies (See, for example, Scher and Daniel, 2005), these subjects are not expected to achieve normal nail plate growth even after the underlying infection (or other cause) is resolved. In these cases, treatment results in partial, but rarely full, plate clearance. Examination of the corresponding photographs appears to bear this out for the predominant number of cases not fully cleared in the 9-12 month post-Tx sample. We do note that only 9% of the sample population (N=9) falls into this senior study subgroup, so a longer study duration (or larger study) with detailed matrix evaluation would be needed to conclusively prove significance of this (highly likely) conclusion. We do note that all of the 9-12 month subgroup achieved at least 36% clearance of the initially compromised areas of the nail plate.

Worksheet#5 examines the dependence of plate clearance on moderate (5-8 J/cm2) and higher (8-12 J/cm2) fluences used in this study. We note that these fluence levels are typical of those employed in treatment of various lesions and removal of tattoos, and, for most subjects under most clinical conditions, these fluence levels result in limited affect, except for minor sensation and sometimes slight erythema, on normal dermal tissues. Interestingly, and somewhat counter-intuitively, the study not only does not reveal an increased efficacy at the higher fluences, it actually suggests a significant decrease in efficacy for those fluences across the sample population. (We note that here 12% of the sample population was excluded due to initial treatment at fluences below 5 J/cm2 and/or treatment at multiple widespread fluence levels.) While further study of this is indicated, we note that the single literature study [Vural, et. Al., 2008] of the effect of a Q-switched 1064 nm laser on cultured T-Rubrum colonies shows a similar (although not necessarily statistically significant) effect: that is, a higher initial rate of colony regrowth for a fluence of 4 J/cm2 than for 8 J/cm2. There are a few possible rationales for this, but, at present, we defer any speculation. We merely point out that there exists a possibility that there might be an optimal fluence level for fungal-cytosis and plate clearance: That is, even neglecting increasing possibility of unwanted side-effect, more fluence may not be more effective. Furthermore, clearly, once the fluence level is sufficient to cause nearly 100% resolution of any infectious agent, more fluence most probably does not lead to a better treatment protocol. Results of the present study do not indicate significantly improved efficacy for treatment fluences above 8 J/cm2, although there also appears to be no significant downside (side-effect) to using treatment fluences at least up to 12 J/cm2, the highest we employed.

Worksheet 6 compares the study subgroup having had only a single treatment (79% of sample) with the subgroup having had multiple (2 or 3) treatments (21% of sample). While the latter subgroup is not large enough to gain high confidence levels for the result, there is no meaningful evidence within this sample population for a finding that successive treatments lead to increased nail plate clearance. In fact

a slight, but not statistically significant reduction of mean clearance levels is evinced. While it would be hard to understand how later treatments could actually reduce efficacy, there are biases in the sampling here; for example, nails showing early strong clearance were not selected for further treatment, biasing the multiple treatment population toward subjects evincing slower clearing and less responsive dystrophies. Clearly, further study is indicated, and one is ongoing, including an assessment to determine whether treatment with 532 nm (the second harmonic) can significantly increase efficacy.

Conceptual Underpinnings and Related Background

Due to the substantially improved efficacy of the Q-Clear™ laser system, we comment on its modality and on the conceptual underpinnings of its likely mechanism of action. For this we assume, as is borne out in the literature (*See Scher and Daniels, 2005*), that the preponderance of the study subjects are nails having dystrophic plates due to underlying infection, predominantly due to fungal infection (onychomychosis) predominantly caused by dermatophytes such as T. rubrum, possibly with smaller contribution due to other infectious organisms.

Nail clearance therefore is predominantly consequential to resolution of the infection. The desired laser action is cytolytic, and selectively so as to minimize collateral damage to dermal tissues, which may cause pain or disrupt the nail matrix or nail bed delaying or preventing healthy plate regrowth. As has been noted (Verul et al, 2008), laser fungal-cytolysis may be due to photothermal or photomechanical/photoacoustic mechanisms. The photothermal mechanisms are due to the absorption of light energy and its concerted conversion into heat resulting in a localized rise in temperature to levels intolerable to the organisms, these levels are thought to be up to 65°C for most fungi (Garcia-Solache and Casadevall, 2010), while the photomechanical/photoacoustic effects are due to photoabsorption resulting in localized pressure increases sufficient to disrupt cellular growth, metabolism, reproduction, and/or colonization. Examples of the latter include shockwave generation and spallation. We note that either photothermal or photomechanical processes can result in ablation, but ablation was not evident under our treatment conditions, and, if any did occur, it was localized to the infectious agent, as dermal ablation was not observed. Generally, the more selective and localized (energetically confined) the process, the greater will be its safety and efficacy. Photoselective processes necessarily start with selective deposition of light energy into the target and specificity requires that the resultant energy deposition remain confined in lethal effect to the target while being tolerable to nearby non-targeted tissue (Anderson and Parrish, 1983). Here we consider the effect on fungal structures, but some of these same considerations apply to selective effects on other organisms.

The general morphology of Eumycetes, or true fungi, is as follows (see for example "Fungi" in Wikipedia, 2011): They are distinguished from plant and animal cells by cell walls made of chitin, a log chain polymer of N-acetylglucosamine, a glucose

derivative, similar in structure to cellulose but having hydrogen-bonded cross-links, which give it increased strength and rigidity, especially in various modified forms.

We note that because both chitin and cellulose encapsulated cells are more rigid and better thermally insulated from their surroundings than membrane encapsulated (animal) cells, they are subject to fracture under certain types of stress and can confine heat for longer periods of time.

The basic fungal unit is the hypha, consisting of one or more cells surrounded by a tubular cell wall. The cells are defined within the structure by internal, often porous, walls, and contain nuclei and various organelles facilitating metabolism and growth. The multicellular hyphae structure, typically 2 to 10 μm in diameter and up to several cm long, is called a mycelium. Hyphae can have various morphologies, but generally grow at their tips and branch and bifurcate creating structures of mycelial cords that can form into networks and webs. When the network of mycelia become large and well enough developed they form colonies often colored and visible to the naked eye. These colonies can form biofilm aggregates and, when extended over nutrients and environmental surfaces, are commonly called molds. In certain cases these fungal aggregates form parasitically on plants and animals where they are capable of exerting enormous local pressures, sufficient to penetrate cell walls and membranes in order to extract nutrients. They also excrete enzymes that can breakdown or assist in the breakdown of cells onto which they attach. This is their role in onychomychosis.

Mechanism of Action

While the parasitic nature of certain fungi is the bane of toenails, their general morphological characteristics make them amenable to selective targeting by laser light. Here we describe the likely etiology of improved selective photolysis using the Q-Clear™ laser system, with its particular laser pulse structure.

Because chitin is long chain and conjugated it has significant absorption in the near IR between 850 nm and 1300 nm (G. Luna-Barcenas, et al). Further, fungal structures often contain pigments that also absorb in the near IR, and less water per unit volume than dermal tissues, giving them a lower heat capacity. These properties, together with their thermally insulating cell wall and relatively rigid structures make both hyphae and the mycelial networks particularly susceptible to violent thermo-mechanical disruption by short (ns duration) 1064 nm laser pulses.

Unfortunately, the dermal structures (hyponychium, matrix, and nail bed) are themselves thermally insulated from above by the nail plate. This means that superficial and volumetric deposition of energy under the nail plate must be minimized to avoid pain and adverse response. For best clinical practice, the aim is to deposit energy selectively and in a time frame that yields maximal disruption to the fungal colony under conditions well tolerated by the surrounding dermal structures. Resolution of the infection may not, and likely does not, require 100%

cytolysis, but merely a sufficient disruption or weakening of the colonized infectious agent so that the body's immune response can resolve the problem. The healthy human body appears to be particularly good at dealing with fungal assaults [Robert and Casadevall, 2009; Garcia-Solache and Casadevall, 2010].

The 1064 nm near IR wavelength of Nd:YAG laser systems is well transmitted through the nail plate, even when somewhat compromised and discolored, and gives rise to the required selective absorption into the hyphae. In dermal tissues, this wavelength has been extensively proved to very well-tolerated even at realtively high fluence levels for both long-pulsed (pulse duration > roughly 50 μs or so and Q-switched (pulse duration 1 – 1000 ns) lasers. At this wavelength the average penetration depth into dermal tissues is several centimeters. This means that light penetrating into tissues beneath the nail plate is absorbed only weakly and into a fairly large tissue volume – made even larger by diffusive scattering as the light penetrates. The ability of these tissues to comply under pressure, to dissipate heat efficiently by thermal conduction, and to undergo a reduced temperature increase even when they do absorb - due to their relatively high heat capacity - makes them more resistant to adverse photothermal and photoacoustic effects. As a result, delivering laser energy in a short timeframe pulses or in bursts of such pulses, having durations less than the timescale for fungal cells to dissipate internally absorbed energy but with low enough fluences and/or sufficent intervals between pulses to permit dermal tissues to dissipate any energy they absorb or that might be conducted into them by adjacent absorbers, is key to providing the widest margin between affecting the targeted fungal structures and adversely affecting the dermal tissues.

A good estimate of the timescale for thermal confinement of heat within the (cylindrical) fungal structures is given by (Anderson and Parrish, 1983):

$$t_R = d^2/16\kappa,$$

where t_R is the thermal relaxation time (half life) and κ is the thermal diffusivity. For most fungi (d = 2 – 10 μm, $\kappa = 10^{-3}$ cm^2/s) the thermal confinement time is

$$t_R \approx 2 – 50 \text{ μs},$$

implying that the laser pulse energy is best delivered in pulses shorter than this.

Recent mycological studies have shown that most fungi do not tolerate temperatures above 50-60°C or so (see Robert and Casadevall, 2009), so thermal confinement alone may well suffice to destroy the colonies even absent significant intracellular photomechanical effects, but certainly photomechanical effects are only of help in breaking up myecelial networks, further improving the likelihood of helping assaulted tissues to overcome the localized infection. Consequently, laser pulses having a temporal format consisting of very short pulses separated by intervals of several tens of microseconds should be nearly optimal in providing pressure and temperature spikes most effective in destroying fungal cells and disrupting fungal colonies while being well-tolerated by the adjacent tissues.

This is exactly what the pulse format designed into Q-Clear™ laser system does, and we believe this has been responsible for its improved efficacy and lower rate of side effect in treating dystrophic toenails, as well as in its proven efficacy in treating its previously cleared indications: tattoos, pigmented and vascular lesions. The Q-Clear™ laser output pulse consists of an envelope of approximately 100 μs with an underlying substructure of up to four sub-pulses, each approximately 7 ns in duration and each having consistent energy (approximately 200 mJ per sub-pulse). This means that the shock and temperature rise induced by these pulses is highly reproducible and why, absent other more strongly absorbing moieties, Q-=Clear™ laser pulses are generally well-tolerated in most healthy tissues. However, when selectively absorbed and thermally confined, local temperatures can build up in and near the absorber during the Q-Clear™ pulse envelope, providing thermal and thermomechanical spikes. The thermal spikes can result in significantly higher local temperatures than would occur had the pulse energy been deposited uniformly over the entire pulse envelope, as is the case with "long pulse" lasers. Such is a likely benefit for eradicating hyphae and other organisms with few micrometer dimension. The ability to induce higher internal temperatures (than would obtain for the same pulse energy evenly distributed over the pulse envelope) at the same overall fluence level leads to the largest temperature differential between the targeted organism and the surrounding tissue.

Because dermal tissues have higher heat capacity and are more efficient in dissipating heat (usually into the vascular system) on the timescale of the pulse envelope, for a given laser pulse energy density (fluence) the temperature rise in the dermal tissues can be lower than for a standard (uniformly distributed) long-pulse laser, while the temperature rise in the targeted organism is higher. This increases the margin between efficacy and unwanted side-effect. We point out that in now over 1 million treatments of tattoos and lesions the Q-Clear™ laser system is yet to have a single significant adverse reaction reported to us. We believe that its unique temporal format is partly responsible for this superb safety record as well as its historic rate of success in treating its previously cleared indications. In the context of the present study, we believe its "double-barreled" capability, providing confined thermo-mechanical shock and localized thermal spikes – both selectively targeted into the fungi, provides a significant advantage over other laser modalities for treating toenails where dystrophy is due to infection by fungi or other agents that absorb 1064 nm light more strongly than surrounding tissues. Given the significant rate of success we have achieved in clearing dystrophic nails, we believe that the benefits of Q-Clear™ laser treatments likely extend, at least to some degree, to toenail dystrophies, beyond onychomychosis, but characterization to specific infectious agents is beyond the scope of the present study.

Conclusion

Our study of 100 Q-Clear™ laser treated subjects has demonstrated substantially effective clearance of dystrophic toenails having a clinically apparent diagnosis of onychomychosis. Statistical analysis of results indicates significant apparent clearing in 95 % of the subjects with an average clearance of affected areas of 56±7

% at a 98% level of confidence. Such high levels of efficacy on a population of this size suggests that the positive effects of the treatment protocol may extend to dystrophies additional to onychomychosis. The protocol employed was extremely well-tolerated by patients, no pain was reported, although some patients reported feeling a low-level sensation on some involved toenails. Reported patient satisfaction was 100%. No significant adverse reactions or responses were observed or reported.

While tracking the specific pathogen initially responsible for the dystrophy was outside the scope of our clinical study, we believe that this study together with independently reported mycological results for 1064 nm Q-switched laser pulses at fluence levels identical to those employed here [Vural, et al., 2008] provide a reasonable basis for allowance of an additional indication for clearance of toenail dystrophies due to mycotic infection (onychomychosis).

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Appendices

Appendix A:

Pre-Treatment and End-of-Study photographs of 100 random subjects from a 12-month duration study on nail clearance following Q-Clear™ laser treatment of initially dystrophic toenails.

Appendix B:

Analysis of data quantified from study photographs in Appendix A

Appendix B:

Analysis of Data
For
A Clinical Study of Q-Clear™ Laser-Treatment of Dystrophic Toenails

Note: Worksheet Tabs Referenced In Study Report Are Indicated in Page Footers (Bottom Left)

Study# - Patient's Initials	Date of Service	Wavelength (nm)	Power Setting J/cm2	Spot Size (mm)	Hertz	Picture #	Comments	Percentage of Clear Nail pre/post Tx
#1 - JT	3/16/10	1064	3.0	5.0	5	#1.1		
	6/15/10	1064	3.0	5.0	5	#1.2	No Treatment	
	9/11/10	1064	3.0	5.0	5	#1.3	No Treatment	
	12/16/10	1064	3.0	5.0	5	#1.4	No Treatmen	72%/100%
#2 - SM	3/11/10	1064	1.8	5.0	1	#2.1		
	6/17/10	1064	11.8	2.5	5	#2.2		
	10/19/10	1064	11.8	2.5	5	#2.3		54%/100%
#3 - BN	3/11/10	1064	3.0	5.0	1	#3.1		
	7/20/10	1064	11.8	2.5	5	#3.2		
	10/19/10	1064	11.8	2.5	5	#3.3		36%/50%
#4 - RS	3/8/10	1064	11.8	2.5	5	#4.1		
	12/10/10	1064	11.8	2.5	5	#4.2	No Treatmen	4%/52%
#5 - RP	3/25/10	1064	5.2	3.5	5	#5.1L	Left	
	7/1/10	1064	5.2	3.5	5	#5.2L	No Treatment	
	11/8/10	1064	5.2	3.5	5	#5.3L	No Treatmen	82%/100%
#6 - RP	3/25/10	1064	5.2	3.5	5	#5.1R	Right	
	7/1/10	1064	5.2	3.5	5	#5.2R	No Treatment	
	11/8/10	1064	5.2	3.5	5	#5.3R	No Treatmen	80%/100%
#7 - LW	3/23/10	1064	5.2	3.5	5	#6.1L	Left	
	6/23/10	1064	11.8	2.5	5	#6.2L		
	9/21/10	1064	11.8	2.5	5	#6.3L		
	3/15/11	532/1064	6.5	3.5	5	#6.4L		5%/58%
#8 - LW	3/23/10	1064	5.2	3.5	5	#6.1R	Right	
	6/23/10	1064	11.8	2.5	5	#6.2R		
	9/21/10	1064	11.8	2.5	5	#6.3R		
	3/15/11	532/1064	6.5	3.5	5	#6.4R		16%/62%
#9 - SH	4/12/10	1064	5.2	3.5	5	#7,1		
	7/15/10	1064					No treatment	
	1/13/11	532/1064	3.9	3.5	5	#7,2		4%/59%
#10 - JS	3/18/10	1064	6.5	3.5	5	#8.1		
	6/18/10	1064				#8.2	No treatment	
	12/10/10	1064	11.8	2.5	5	#8.3		3%/38%
#11 - JO	3/22/10	1064	3.9	3.5	5	#9.1		
	8/30/10	1064					No treatmen	72%/97%
#12 - LH	3/23/10	1064	5.2	3.5	5	#10.1		
	6/24/10	1064	7.0	2.5	5	#10.2		
	9/23/10	1064	7.5	3.5	5	#10.3		0%/30%
#13 - AM	3/22/10	1064	5.2	3.5	5	#11.1L	Left	
	6/22/10	1064				#11.2L	No treatment	
	8/12/10	1064				#11.3L	No treatmen	0%/91%
#14 - AM	3/22/10	1064	5.2	3.5	5	#11.1	Right	
	6/22/10	1064				#11.2	No treatment	
	8/12/10	1064				#11.3	No treatmen	0%/92%

#15 - MD	3/26/10	1064	5.2	3.5	5	#12.1		
	7/19/10	1064	11.8	2.5	5	#12.2		77%/98%
#16 - ES	4/7/10	1064	5.2	3.5	5	#13.1		
	7/1/10					#13.2	No treatment	
	10/7/10					#13.3	No treatmen	79%/100%
#17 - MH	4/7/10	1064	5.2	3.5	3	#14.1		
	10/6/10	1064	11.8	2.5	5	#14.2		52%/100%
#18 - DT	4/14/10	1064	6.5	3.5	2	#15.1		
	7/14/10	1064	11.8	2.5	3	#15.2		76%/97%
#19 - LH	4/21/10	1064	5.2	3.5	5	#16.1		
	11/11/10	1064	11.8	2.5	5	#16.2		28%/100%
#20 - ST	4/28/10	1064	11.8	2.5	3	#17.1		
	8/4/10	1064	11.8	2.5	3	#17.2		
	10/13/10	1064	11.8	2.5	3	#17.3		23%/86%
#21 - BB	5/4/10	1064	11.8	2.5	3	#18.1		
	8/4/10	1064	11.8	2.5	3	#18.2		
	11/3/10	1064	11.8	2.5	3	#18.3		58%/93%
#22 - SV	5/10/10	1064	7.0	2.5	1	#19.1		
	8/16/10					#19.2	No treatmen	66%/97%
#23 - JJ	5/18/10	1064	11.8	2.5	3	#20.1		
	8/25/10	1064	11.8	2.5	3	#20.2		
	11/30/10	1064	11.8	2.5	3	#20.3		93%/95%
#24 - SC	5/20/10	1064	9.4	2.5	2	#21.2L	Left	
	9/16/10					#21.3L	No treatmen	85%/100%
#25 - SC	5/20/10	1064	9.4	2.5	2	#21.1	Right	
	9/16/10					#21.4	No treatmen	64%/87%
#26 - RS	5/24/10	1064	9.4	2.5	2	#22.1		
	9/20/10					#22.2	No treatment	
	1/17/11					#22.3	No treatmen	0%/42%
#27 - FA	5/27/10	1064	11.8	2.5	3	#23.1		
	9/2/10					#23.2	No treatment	
	12/9/10					#23.3	No treatmen	57%/85%
#28 - TR	6/2/10	1064	11.8	2.5	3	#24.1		
	9/8/10					#24.2	No treatmen	88%/97%
#29 - JA	6/3/10	1064	11.8	2.5	3	#25.1		
	9/2/10					#25.2	No treatment	
	12/2/10					#25.3	No treatmen	94%/95%
#30 - DS	6/11/10	1064	9.4	2.5	2	#26.1		
	10/13/10					#26.2	No treatment	
	2/18/11					#26.3	No treatmen	21%/92%
#31 -DJ	6/25/10	1064	11.8	2.5	3	#27.1		

	10/1/10					#27.2	No treatmen	37%/96%
#32 - JM	6/29/10	1064	11.8	2.5	3	#28.1		
	9/28/10					#28.2	No treatment	
	12/28/10					#28.3	No treatmen	33%/88%
#33 - CK	6/30/10	1064	9.4	2.5	2	#29.1		
	10/27/10					#29.2	No treatment	
	3/1/11	532/1064	11.8	2.5	4	#29.3		20%/50%
#34 - KW	7/22/10	1064	11.8	2.5	3	#30.1		
	10/19/10	1064	11.8	2.5	3	#30.2		90%/97%
#35 - FB	7/28/10	1064	5.2	2.5	4	#31.1		
	11/3/10					#31.2	No treatment	
	2/9/11	1064	6.5	3.5	4	#31.3		19%/92%
#36 - MR	8/11/10	1064	7.0	2.5	3	#32.1		
	11/8/10					#32.2	No treatment	
	2/10/11					#32.3	No treatmen	29%/80%
#37 - TP	8/31/10	1064	11.8	2.5	5	#33.1		
	12/21/10		11.8	2.5	5	#33.2		
	2/22/11	532/1064	11.8	3.0	4	#33.3		38%/77%
#38 - MC	9/23/10	1064	3.6	5.0	3	#34.1		
	3/24/11	1064	3.0	5.0	5	#34.2		92%/96%
#39 - DP	10/1/10	1064	11.8	3.0	5	#35.1		
	1/14/11	1064	11.8	3.0	4	#35.2		0%/63%
#40 - SL	10/26/10	1064	11.8	3.0	5	#36.1		
	2/22/11					#36.2	No treatmen	88%/97%
#41 - JS	10/26/10	1064	6.5	3.5	3	#37.1L	Left	
	2/22/11	532/1064	6.5	3.5	3	#37.2L		41%/95%
#42 - JS	10/26/10	1064	6.5	3.5	3	#37.3	Right	
	2/22/11	532/1064	6.5	3.5	3	#37.4		55%/98%
#43 - CD	11/30/10	1064	11.8	2.5	5	#38.1		
	4/5/11	1064	6.5	3.5	5	#38.2		36%/98%
#44 - JB	5/12/10	1064	7.0	2.5	2	#39.1		
	10/28/10					#39.2	No Treatmen	78%/91%
#45 - MC	8/31/10	1064	11.8	2.5	3	#40.1		
	11/30/10					#40.2	No Treatment	
	2/22/11	532/1064	6.5	3.5	3	#40.3		
	5/10/11	1064	11.8	2.5	5	#40.4		0%/65%
#46 - KM	4/26/10	1064	9.4	2.5	1	#41.1		
	7/26/10						No Treatmen	0%/34%
#47 - MM	4/13/10	1064	5.2	3.5	5	#42.1		
	11/16/10						No Treatment	
	2/24/11	532/1064	3.9	3.5	5	#42.3		25%/57%

#48 - ES	5/4/10	1064	11.8	2.5	3	#43.1		
	8/3/10					#43.2	No Treatment	
	2/22/11	532/1064	6.5	3.5	3	#43.3		21%/54%
#49 - RV	6/21/10	1064	11.8	2.5	3	#44.1		
	9/20/10	1064	11.8	2.5	3	#44.2		
	3/14/11	532/1064	6.5	3.5	5	#44.3		27%/70%
#50 - HC	6/22/10	1064	11.8	2.5	3	#45.1		
	9/21/10	1064	11.8	2.5	3	#45.2		
	12/14/10	532/1064	7.0	2.5	1	#45.3		0%/5%
#51 - BH	6/24/10	1064	11.8	2.5	4	#46.1	3rd toe	
	9/27/10					#46.2	No Treatment	
	12/20/10					#46.3	No Treatmen	67%/71%
#52 -MC	6/29/10	1064	11.8	2.5	1	#47.1		
	9/27/10					#47.2	No Treatment	
	12/20/10	1064	11.8	2.5	1	#47.3		
	3/21/11	532/1064	6.5	3.5	3	#47.4		10%/23%
#53 - EM	6/29/10	1064	11.8	2.5	3	#48.1		
	9/28/10					#48.2	No Treatment	
	12/14/10	1064	11.8	2.5	3	#48.3		11%/26%
#54 - WW	7/15/10	1064	11.8	2.5	3	#49.1		
	11/9/10					#49.2	No Treatment	
	2/1/11	1064	6.5	3.5	5	#49.3		
	5/5/11	1064	11.8	2.5	5	#49.4		15%/48%
#55 - CL	7/26/10	1064	11.8	2.5	3	#50.1		
	11/8/10					#50.2	No Treatmen	18%/42%
#56 - MB	7/27/10	1064	9.4	2.5	5	#51.1		
	10/26/10					#51.2	No Treatment	
	1/25/11	532/1064	3.0	5.0	5	#51.3		9%/29%
#57 - MC	7/29/10	1064	11.8	2.5	3	#52.1		
	10/26/10					#52.2	No Treatment	
	1/25/11					#52.3	No Treatmen	84%/89%
#58 - JM	7/29/10	1064	11.8	2.5	3	#53.1L	Left	
	10/21/10						No Treatmen	94%/98%
#59 - JM	7/29/10	1064	11.8	2.5	3	#53.3	Right	
	10/21/10						No Treatmen	86%/97%
#60 - JC	8/10/10	1064	7.0	2.5	5	#54.1L	Left	
	11/9/10	1064	7.5	3.5	4	#54.2L		
	2/10/11					#54.3L	No Treatmen	9%/59%
#61 - JC	8/10/10	1064	7.0	2.5	5	#54.4	Right	
	11/9/10	1064	7.5	3.5	4	#54.5		
	2/10/11					#54.6	No Treatmen	32%/55%
#62 - RG	8/10/10	1064	7.0	2.5	5	#55.1		
	11/11/10	1064	11.8	2.5	5	#55.2		
	2/10/11	1064	6.5	3.5	5	#55.3		27%/41%

#63 - BK	9/13/10	1064	11.8	2.5	5	#56.1		
	12/30/10	532/1064	3.9	3.5	2	#56.2		
	4/7/11	1064	6.5	3.5	5	#56.3		21%/92%
#64 - GF	9/14/10	1064	11.8	2.5	5	#57.1		
	12/9/10					#57.2	No Treatmen	92%/95%
#65 - MM	10/26/10	1064	11.8	2.5	5	#58.1L	Left	
	2/22/11	532/1064	6.5	3.5	5	#58.2L		38%/56%
#66 - MM	10/26/10	1064	11.8	2.5	5	#58.3	Right	
	2/22/11	532/1064	6.5	3.5	5	#58.4		58%/76%
#67 - LR	11/30/10	1064	11.8	2.5	5	#59.1		
	3/29/11	1064	11.8	2.5	5	#59.2		53%/71%
#68 - JF	12/9/10	1064	11.8	2.5	5	#60.1		
	4/28/11	1064	11.8	2.5	5	#60.2		35%/52%
#69 - WH	8/25/10	1064	11.8	2.5	3	#61.1L	Left	
	11/29/10	1064	11.8	2.5	3	#61.2L		17%/26%
#70 - WH	8/25/10	1064	11.8	2.5	3	#61.3	Right	
	11/29/10	1064	11.8	2.5	3	#61.4		37%/84%
#71 - WY	8/2/10	1064	11.8	2.5	3	#62.1		
	12/6/10					#62.2	No Treatmen	17%/53%
#72 - JV	9/1/10	1064	11.8	2.5	3	#63.1L	Left	
	12/1/10	1064	11.8	2.5	3	#63.2L		40%/30%
#73 - JV	9/1/10	1064	11.8	2.5	3	#63.3	Right	
	12/1/10	1064	11.8	2.5	3	#63.4		48%/96%
#74 - SE	3/17/10	1064	3.0	5.0	5	#64.1		
	9/28/10	1064	11.8	2.5	3	#64.2		0%/11%
#75 - MH	4/6/10	1064	5.2	3.5	3	#65.1		
	7/6/10					#65.2	No Treatment	
	10/5/10	1064	11.8	2.5	3	#65.3		65%/98%
#76 - DR	4/27/10	1064	11.8	2.5	3	#66.1		
	7/27/10	1064	11.8	2.5	3	#66.2		
	11/2/10	1064	11.8	2.5	3	#66.3		11%/52%
# 77 - PW	5/11/10	1064	11.8	2.5	3	#67.1		
	8/11/10	1064	11.8	2.5	3	#67.2		0%/81%
#78 - CT	5/14/10	1064	11.8	2.5	3	#68.1		
	8/27/10	1064	11.8	2.5	3	#68.2		
	11/19/10	1064	11.8	2.5	3	#68.3		0%/23%
#79 - WF	10/28/10	1064	11.8	2.5	5	#69.1		
	2/18/11					#69.2	No Treatmen	53%/91%
#80 - GS	9/7/10	1064	11.8	2.5	5	#70.1		
	1/5/11	1064	11.8	2.5	5	#70.2		3%/3%
#81 - DS	5/25/10	1064	11.8	2.5	3	#71.1		
	9/13/10	1064	11.8	2.5	3	#71.2		14%/24%

#82 - GH	5/26/10	1064	11.8	2.5	3	#72.1		
	8/27/10	1064	11.8	2.5	3	#72.2		91%/97%
#83 - SP	5/26/10	1064	11.8	2.5	3	#73.1		
	9/3/10	1064	11.8	2.5	3	#73.2		3%/36%
#84 - MS	6/8/10	1064	11.8	2.5	3	#74.1		
	9/8/10	1064	11.8	2.5	3	#74.2		
	12/8/10	1064	11.8	2.5	3	#74.3		21%/49%
#85 - DC	6/15/10	1064	11.8	2.5	3	#75.1		
	10/11/10	1064	11.8	2.5	3	#75.2		56%/45%
#86 - LZ	6/15/10	1064	11.8	2.5	3	#76.1		
	9/15/10	1064	11.8	2.5	3	#76.2		81%/100%
#87 - DS	7/6/10	1064	11.8	2.5	3	#77.3	Left	
	10/5/10	1064	11.8	2.5	3	#77.2L		69%/83%
#88 - DS	7/6/10	1064	11.8	2.5	3	#77.1l	Right	
	10/5/10	1064	11.8	2.5	3	#77.4		77%/68%
# 89 - CR	7/14/10	1064	11.8	2.5	3	#78.1L	Left	
	10/29/10	1064	11.8	2.5	3	#78.2L		27%/86%
#90 - CR	7/14/10	1064	11.8	2.5	3	#78.3	Right	
	10/29/10	1064	11.8	2.5	3	#78.4		57%/79%
#91 - VC	8/3/10	1064	11.8	2.5	3	#79.1L	Left	
	11/2/10	1064	11.8	2.5	3	#79.2L		0%/37%
#92 - VC	8/3/10	1064	11.8	2.5	3	#79.3	Right	
	11/2/10	1064	11.8	2.5	3	#79.4		28%/56%
#93 - TD	8/4/10	1064	11.8	2.5	3	#80.1		
	11/3/10	`	11.8	2.5	3	#80.2		0%/32%
# 94 - PS	9/15/10	1064	6.5	3.5	3	#81.1L	Left	
	12/15/10	1064	11.8	2.5	3	#81.2L		58%/83%
# 95 -PS	9/15/10	1064	6.5	3.5	3	#81.3	Right	
	12/15/10	1064	11.8	2.5	3	#81.4		48%/68%
#96 - SR	11/16/10	1064	11.8	2.5	5	#82.1		
	3/15/10	1064	11.8	2.5	5	#82.2		79%/88%
# 97 - LD	1/17/11	1064	6.5	3.5	5	#83,1r	Right	
	5/16/11					#83.2r	No treatmen	24%/91%
#98 - LD	1/17/11	1064	6.5	3.5	5	#83.1l	Left	
	5/16/11					#83.2l	No treatmen	12%/95%
#99 - KS	1/18/11	1064	6.5	3.5	5	#84.1		
	5/16/11	1064	11.8	2.5	5	#84.2		60%/60%
#100 - DD	10/20/10	1064	11.8	2.5	5	#85.1		
	1/20/11	1064/532	6.5	3.5	5	#85.2		0%/47%

Case #	STUDY PERIOD Months Post Initial Tx	Use of 532 nm	Treatment Fluence Level: (L)ow<5, (M)edium 5-8, (H)igh 8-12, (V)ariable	Number of Tx- prior to last observation	Clearance of Dystrophy (post-pre)÷(1-pre)
1	9.	0	L	1	100%
2	7.	0	V	2	100%
3	7.	0	V	2	22%
4	9.	0	H	1	50%
5	7.5	0	M	1	100%
6	7.5	0	M	1	100%
7	12.	Last Only	V	3	56%
8	12.	Last Only	V	3	55%
9	9.	Last Only	M	1	57%
10	9.	0	M	1	36%
11	5.	0	L	1	89%
12	6.	0	M	2	30%
13	4.5	0	M	1	91%
14	4.5	0	M	1	92%
15	4.	0	M	1	91%
16	6.	0	M	1	100%
17	6.	0	M	1	100%
18	3.	0	M	1	88%
19	7.7	0	M	1	100%
20	5.5	0	H	2	82%
21	6.	0	H	2	83%
22	3.	0	M	1	91%
23	6.5	0	H	2	29%
24	4.	0	H	1	100%
25	4.	0	H	1	64%
26	7.8	0	H	1	42%
27	6.5	0	H	1	65%
28	3.	0	H	1	75%
29	6.	0	H	1	17%
30	8.	0	H	1	90%
31	3.2	0	H	1	94%
32	6.	0	H	1	82%
33	8.	0	H	1	38%
34	3.	0	H	1	70%
35	6.5	0	M	1	90%
36	6.	0	M	1	72%
37	5.8	0	H	2	63%
38	6.	0	L	1	50%
39	3.5	0	H	1	63%
40	4.	0	H	1	75%
41	4.	Last	M	1	92%
42	4.	Last	M	1	96%
43	4.2	0	H	1	97%
44	5.5	0	M	1	59%
45	8.4	2.8 post	V	2	65%
46	3.	0	H	1	34%

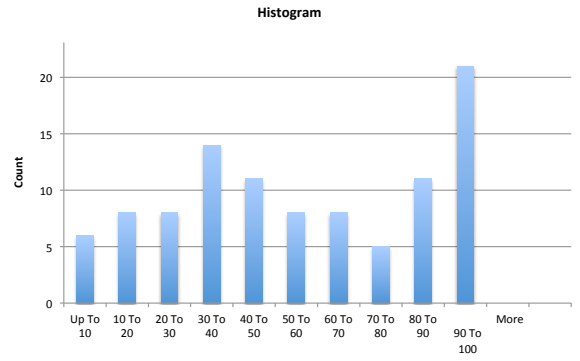
47	10.4	Last	M	1	43%
48	9.6	Last	H	1	42%
49	8.8	Last	H	2	59%
50	5.8	Last	H	2	5%
51	6.	0	H	1	12%
52	8.8	Last	H	2	14%
53	5.5	0	H	1	17%
54	9.7	0	V	2	39%
55	3.5	0	H	1	29%
56	6.	Last	H	1	22%
57	6.	0	H	1	31%
58	2.8	0	H	1	67%
59	2.8	0	H	1	79%
60	6.	0	M	2	55%
61	6.	0	M	2	34%
62	6.	0	M,H	2	19%
63	6.8	3.2 post	V	2	90%
64	2.8	0	H	1	37%
65	4.	Last	H	1	29%
66	4.	Last	H	1	43%
67	4.	0	H	1	38%
68	5.6	0	H	1	25%
69	3.	0	H	1	11%
70	3.	0	H	1	75%
71	4.	0	H	1	43%
72	3.	0	H	1	-17%
73	3.	0	H	1	92%
74	6.2	0	L	1	11%
75	6.	0	M	1	94%
76	6.2	0	H	2	46%
77	3.	0	H	1	81%
78	6.	0	H	2	23%
79	3.8	0	H	1	81%
80	4.	0	H	1	0%
81	3.7	0	H	1	12%
82	3.	0	H	1	67%
83	3.3	0	H	1	34%
84	6.	0	H	2	35%
85	4.	0	H	1	-25%
86	3.	0	H	1	100%
87	3.	0	H	1	45%
88	3.	0	H	1	-39%
89	3.2	0	H	1	81%
90	3.2	0	H	1	51%
91	3.	0	H	1	37%
92	3.	0	H	1	39%
93	3.	0	H	1	32%
94	3.	0	M	1	60%
95	3.	0	M	1	38%
96	4.	0	H	1	43%
97	4.	0	M	1	88%
98	4.	0	M	1	94%
99	4.	0	M	1	0%
100	3.	Last	H	1	47%

Histogram	
No# of valid cases	100

Results for layer #1

Frequency distribution of All Tx

All Tx	Count	Cumulative Co	Percent	Cumulative Percent
Up To 10	6.	6.	0.06	0.06
10 To 20	8.	14.	0.08	0.14
20 To 30	8.	22.	0.08	0.22
30 To 40	14.	36.	0.14	0.36
40 To 50	11.	47.	0.11	0.47
50 To 60	8.	55.	0.08	0.55
60 To 70	8.	63.	0.08	0.63
70 To 80	5.	68.	0.05	0.68
80 To 90	11.	79.	0.11	0.79
90 To 100	21.	100.	0.21	1.
More	0.E+0	100.	0.E+0	1.



Note: At Entry (Pre-Tx#1) All Subjects Are at 0% Clearance, by Definition of Metric.

Statistics

Alpha value (for confidence interval)	0.02		

Variable #1 (All Tx)

Count	100	Skewness	-0.1173
Mean	56.21752	Skewness Standard Error	0.23895
Mean LCL	48.98626	Kurtosis	1.8285
Mean UCL	63.44877	Kurtosis Standard Error	0.46393
Variance	935.21168	Alternative Skewness (Fisher's)	-0.11909
Standard Deviation	30.58123	Alternative Kurtosis (Fisher's)	-1.16977
Mean Standard Error	3.05812	Coefficient of Variation	0.54398
Minimum	0.E+0	Mean Deviation	26.54253
Maximum	100.	Second Moment	925.85956
Range	100.	Third Moment	-3,304.58012
Sum	5,621.75158	Fourth Moment	1,567,422.06624
Sum Standard Error	305.81231	Median	55.36726
Total Sum Squares	408,626.86431	Median Error	0.38328
Adjusted Sum Squares	92,585.9562	Percentile 25% (Q1)	34.
Geometric Mean	41.47678	Percentile 75% (Q2)	88.15789
Harmonic Mean	41.29189	IQR	54.15789
Mode	100.	MAD	25.86585

One-Sample z-Test

	All Tx
Mean	56.21752
Variance	935.21168
Sample size	100
p-level	0.02
Hypothesized Population Mean	56.22
Population Variance	935.21
Mean Difference	-0.00248
Mean Difference - 98% LCL	-7.11674
Mean Difference - 98% UCL	7.11177
Standard Error	3.05812
z	-0.00081
P(Z<=z) - One-tailed distribution	0.49968
z Critical Value - One-tailed distributic	2.05375
P(Z<=z) - Two-tailed distribution	0.99935
z Critical Value - Two-tailed distributic	2.32635

56±7% Clearance of dystrophic nails achieved within Study Population

Significance and Confidence 98%

Normality Test

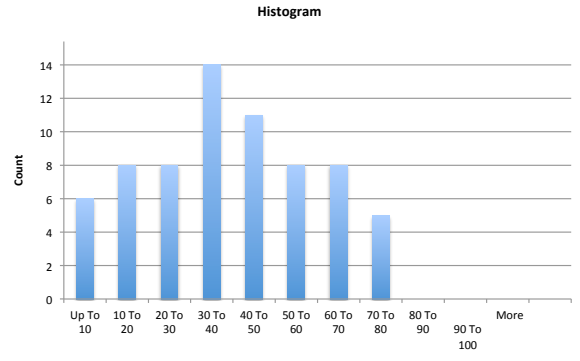
Data with >80% "Cured Outliers" Stripped is nearyl Normal - This verifies statistical assumptions and significance (p-level) and confidence (alpha value) levels for data distribution

Histogram	
No# of valid cases	68

Results for layer #1

Frequency distribution of Clearance of Dystrophy<80

Clearance of I	Count	Cumulative Co	Percent	Cumulative Percent
Up To 10	6.	6.	0.08824	0.08824
10 To 20	8.	14.	0.11765	0.20588
20 To 30	8.	22.	0.11765	0.32353
30 To 40	14.	36.	0.20588	0.52941
40 To 50	11.	47.	0.16176	0.69118
50 To 60	8.	55.	0.11765	0.80882
60 To 70	8.	63.	0.11765	0.92647
70 To 80	5.	68.	0.07353	1.
80 To 90	0.E+0	68.	0.E+0	1.
90 To 100	0.E+0	68.	0.E+0	1.
More	0.E+0	68.	0.E+0	1.



Statistics

Alpha value (for confidence interval)	0.02		

Variable #1 (Clearance of Dystrophy<80%)

Count	68	Skewness	-0.12273
Mean	39.30918	Skewness Standard Error	0.28646
Mean LCL	33.14227	Kurtosis	2.20345
Mean UCL	45.47609	Kurtosis Standard Error	0.54856
Variance	455.28747	Alternative Skewness (Fisher's)	-0.12552
Standard Deviation	21.33747	Alternative Kurtosis (Fisher's)	-0.76467
Mean Standard Error	2.58755	Coefficient of Variation	0.54281
Minimum	0.E+0	Mean Deviation	17.2229
Maximum	78.57143	Second Moment	448.59207
Range	78.57143	Third Moment	-1,166.10691
Sum	2,673.02445	Fourth Moment	443,411.47483
Sum Standard Error	175.95326	Median	38.64253
Total Sum Squares	135,578.66813	Median Error	0.39327
Adjusted Sum Squares	30,504.26074	Percentile 25% (Q1)	25.
Geometric Mean	28.52147	Percentile 75% (Q2)	57.29167
Harmonic Mean	32.80652	IQR	32.29167
Mode	0.E+0	MAD	16.48352

One-Sample z-Test

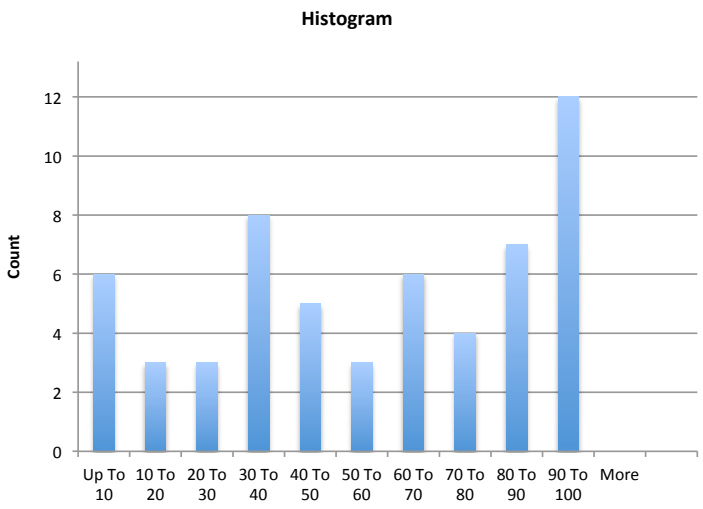
	Clearance of Dystrophy<80%
Mean	39.30918
Variance	455.28747
Sample size	68
p-level	0.02
Hypothesized Population Mean	39.
Population Variance	455.
Mean Difference	0.30918
Mean Difference - 98% LCL	-5.70845
Mean Difference - 98% UCL	6.32682
Standard Error	2.58673
z	0.11953
P(Z<=z) - One-tailed distribution	0.45243
z Critical Value - One-tailed distributic	2.05375
P(Z<=z) - Two-tailed distribution	0.90486
z Critical Value - Two-tailed distributic	2.32635

Histogram	
No# of valid cases	57

Results for layer #1

Frequency distribution of T<6 months

T<6 months	Count	Cumulative Count	Percent	Cumulative Percent
Up To 10	6.	6.	0.10526	0.10526
10 To 20	3.	9.	0.05263	0.15789
20 To 30	3.	12.	0.05263	0.21053
30 To 40	8.	20.	0.14035	0.35088
40 To 50	5.	25.	0.08772	0.4386
50 To 60	3.	28.	0.05263	0.49123
60 To 70	6.	34.	0.10526	0.59649
70 To 80	4.	38.	0.07018	0.66667
80 To 90	7.	45.	0.12281	0.78947
90 To 100	12.	57.	0.21053	1.
More	0.E+0	57.	0.E+0	1.



Statistics

Alpha value (for confidence interval)	0.02		

Variable #1 (T<6 months)

Count	57	Skewness	-0.34806
Mean	56.56613	Skewness Standard Error	0.31073
Mean LCL	46.57914	Kurtosis	1.90626
Mean UCL	66.55312	Kurtosis Standard Error	0.59015
Variance	991.29973	Alternative Skewness (Fisher's)	-0.35754
Standard Deviation	31.48491	Alternative Kurtosis (Fisher's)	-1.08298
Mean Standard Error	4.17028	Coefficient of Variation	0.5566
Minimum	0.E+0	Mean Deviation	27.38642
Maximum	100.	Second Moment	973.90851
Range	100.	Third Moment	-10,578.76252
Sum	3,224.26955	Fourth Moment	1,808,084.35836
Sum Standard Error	237.70588	Median	62.90323
Total Sum Squares	237,897.24343	Median Error	0.69229
Adjusted Sum Squares	55,512.78493	Percentile 25% (Q1)	34.76546
Geometric Mean	37.73914	Percentile 75% (Q2)	87.99342
Harmonic Mean	44.20163	IQR	53.22796
Mode	0.E+0	MAD	25.90323

One-Sample z-Test

	T<6 months
Mean	56.56613
Variance	991.29973
Sample size	57
p-level	0.02
Hypothesized Population Mean	56.57
Population Variance	991.3
Mean Difference	-0.00387
Mean Difference - 98% LCL	-9.70539
Mean Difference - 98% UCL	9.69765
Standard Error	4.17028
z	-0.00093
P(Z<=z) - One-tailed distribution	0.49963
z Critical Value - One-tailed distributi	2.05375
P(Z<=z) - Two-tailed distribution	0.99926
z Critical Value - Two-tailed distributi	2.32635

Note: At Entry (Pre-Tx#1) All Subjects Are at 0% Clearance, by Definition of Metric.

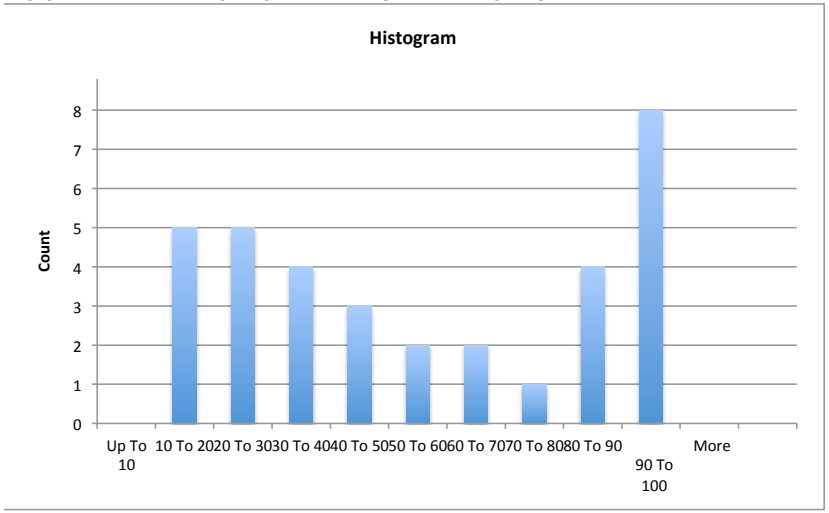
Histogram

No# of valid cases	34

Results for layer #1

Frequency distribution of 6≤T<9 months

6≤T<9 months	Count	Cumulative Co	Percent	Cumulative Percent
Up To 10	0.E+0	0.E+0	0.E+0	0.E+0
10 To 20	5.	5.	0.14706	0.14706
20 To 30	5.	10.	0.14706	0.29412
30 To 40	4.	14.	0.11765	0.41176
40 To 50	3.	17.	0.08824	0.5
50 To 60	2.	19.	0.05882	0.55882
60 To 70	2.	21.	0.05882	0.61765
70 To 80	1.	22.	0.02941	0.64706
80 To 90	4.	26.	0.11765	0.76471
90 To 100	8.	34.	0.23529	1.
More	0.E+0	34.	0.E+0	1.



Alpha value (for confidence interval)	0.02		

Variable #1 (6≤T<9 mos)

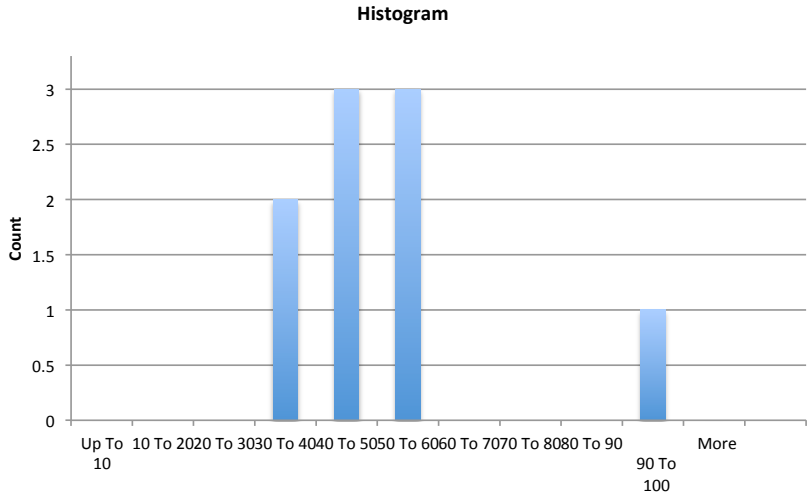
Count	34	*Skewness*	0.11439
Mean	56.47924	*Skewness Standard Error*	0.39102
Mean LCL	43.02017	*Kurtosis*	1.47989
Mean UCL	69.93831	*Kurtosis Standard Error*	0.71772
Variance	1,030.44336	*Alternative Skewness (Fisher's)*	0.11974
Standard Deviation	32.10052	*Alternative Kurtosis (Fisher's)*	-1.57029
Mean Standard Error	5.50519	*Coefficient of Variation*	0.56836
Minimum	11.	*Mean Deviation*	28.63308
Maximum	100.	*Second Moment*	1,000.1362
Range	89.	*Third Moment*	3,618.07437
Sum	1,920.29416	*Fourth Moment*	1,480,288.67218
Sum Standard Error	187.17659	*Median*	52.47253
Total Sum Squares	142,461.38572	*Median Error*	1.1833
Adjusted Sum Squares	34,004.63091	*Percentile 25% (Q1)*	29.28571
Geometric Mean	46.09396	*Percentile 75% (Q2)*	89.99844
Harmonic Mean	35.87448	*IQR*	60.71272
Mode	100.	*MAD*	30.54602

No# of valid cases	9

Results for layer #1

Frequency distribution of T≥9 mos

T?9 mos	Count	Cumulative Cc	Percent	Cumulative Percent
Up To 10	0.E+0	0.E+0	0.E+0	0.E+0
10 To 20	0.E+0	0.E+0	0.E+0	0.E+0
20 To 30	0.E+0	0.E+0	0.E+0	0.E+0
30 To 40	2.	2.	0.22222	0.22222
40 To 50	3.	5.	0.33333	0.55556
50 To 60	3.	8.	0.33333	0.88889
60 To 70	0.E+0	8.	0.E+0	0.88889
70 To 80	0.E+0	8.	0.E+0	0.88889
80 To 90	0.E+0	8.	0.E+0	0.88889
90 To 100	1.	9.	0.11111	1.
More	0.E+0	9.	0.E+0	1.



Alpha value (for confiden	0.02

Variable #1 (9≤T≤12 mos)

Count	9	Skewness	1.73547
Mean	53.02087	Skewness Standard Error	0.63246
Mean LCL	34.42643	Kurtosis	5.13319
Mean UCL	71.61532	Kurtosis Standard Error	0.91856
Variance	370.91466	Alternative Skewness (Fisher's)	2.10371
Standard Deviation	19.25914	Alternative Kurtosis (Fisher's)	5.20607
Mean Standard Error	6.41971	Coefficient of Variation	0.36324
Minimum	36.08247	Mean Deviation	12.39101
Maximum	100.	Second Moment	329.70192
Range	63.91753	Third Moment	10,389.63069
Sum	477.18787	Fourth Moment	557,994.64346
Sum Standard Error	57.77743	Median	50.
Total Sum Squares	28,268.23516	Median Error	2.68197
Adjusted Sum Squares	2,967.3173	Percentile 25% (Q1)	41.99578
Geometric Mean	50.62725	Percentile 75% (Q2)	56.91612
Harmonic Mean	48.78348	IQR	14.92034
Mode	#N/A	MAD	7.33333

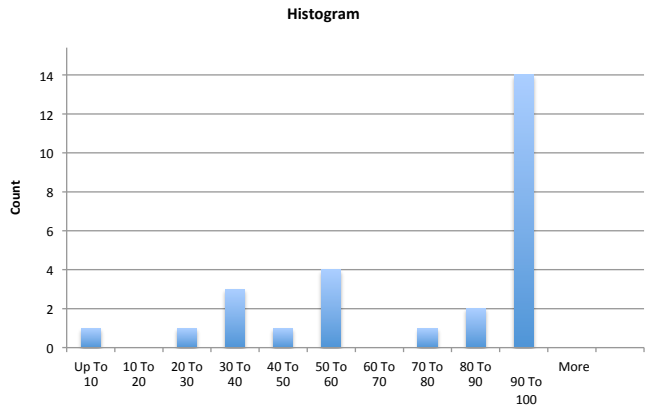
Histogram

No# of valid cases	27

Results for layer #1

Frequency distribution of Medium (5-8 J/cm2)

Medium	(5 Count	Cumulative Co	Percent	Cumulative Percent
Up To 10	1.	1.	0.03704	0.03704
10 To 20	0.E+0	1.	0.E+0	0.03704
20 To 30	1.	2.	0.03704	0.07407
30 To 40	3.	5.	0.11111	0.18519
40 To 50	1.	6.	0.03704	0.22222
50 To 60	4.	10.	0.14815	0.37037
60 To 70	0.E+0	10.	0.E+0	0.37037
70 To 80	1.	11.	0.03704	0.40741
80 To 90	2.	13.	0.07407	0.48148
90 To 100	14.	27.	0.51852	1.
More	0.E+0	27.	0.E+0	1.



Alpha value (for confidence interval)	0.02

Variable #1 (Medium (5-8 J/cm2))

Count	27	Skewness	-0.94129
Mean	73.72828	Skewness Standard Error	0.43095
Mean LCL	60.29754	Kurtosis	2.80387
Mean UCL	87.15903	Kurtosis Standard Error	0.77402
Variance	792.75822	Alternative Skewness (Fisher's)	-0.99759
Standard Deviation	28.15596	Alternative Kurtosis (Fisher's)	0.02203
Mean Standard Error	5.41862	Coefficient of Variation	0.38189
Minimum	0.E+0	Mean Deviation	24.24404
Maximum	100.	Second Moment	763.39681
Range	100.	Third Moment	-19,854.14243
Sum	1,990.66368	Fourth Moment	1,634,024.79943
Sum Standard Error	146.30267	Median	90.12346
Total Sum Squares	167,379.9319	Median Error	1.30697
Adjusted Sum Squares	20,611.71383	Percentile 25% (Q1)	56.70501
Geometric Mean	61.2687	Percentile 75% (Q2)	94.62753
Harmonic Mean	68.59835	IQR	37.92251
Mode	100.	MAD	9.87654

Sensitivity Test

90-100% Outliers stripped

Alpha value (for confidence interval)	0.02

Variable #1 (0)

Count	12	Skewness	0.46202
Mean	54.94788	Skewness Standard Error	0.58177
Mean LCL	39.36537	Kurtosis	2.06004
Mean UCL	70.53038	Kurtosis Standard Error	0.91655
Variance	394.39528	Alternative Skewness (Fisher's)	0.53082
Standard Deviation	19.85939	Alternative Kurtosis (Fisher's)	-0.76016
Mean Standard Error	5.73291	Coefficient of Variation	0.36142
Minimum	30.	Mean Deviation	15.618
Maximum	88.15789	Second Moment	361.52901
Range	58.15789	Third Moment	3,175.97151
Sum	659.37453	Fourth Moment	269,253.85553
Sum Standard Error	68.79494	Median	56.11836
Total Sum Squares	40,569.57899	Median Error	2.07417
Adjusted Sum Squares	4,338.34812	Percentile 25% (Q1)	38.46154
Geometric Mean	51.74643	Percentile 75% (Q2)	71.83099
Harmonic Mean	48.75643	IQR	33.36945
Mode	#N/A	MAD	16.68472

Histogram

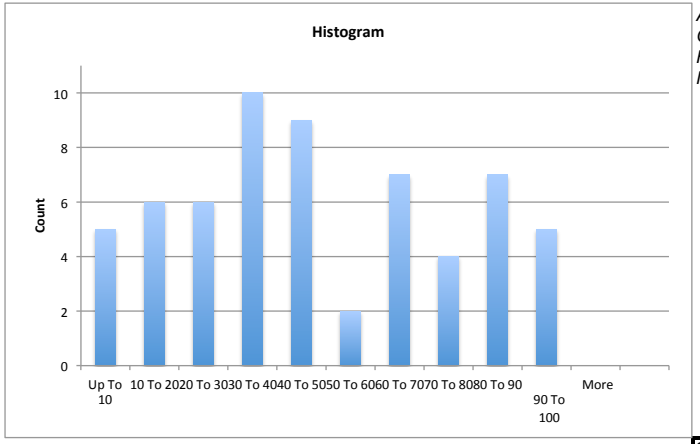
No# of valid cases	61

Alpha value (for confidence interval) 0.02

Results for layer #1

Frequency distribution of High (>8-12 J/cm2)

High	*(: Count*	*Cumulative C*	*Percent*	*Cumulative Percent*
Up To 10	5.	5.	0.08197	0.08197
10 To 20	6.	11.	0.09836	0.18033
20 To 30	6.	17.	0.09836	0.27869
30 To 40	10.	27.	0.16393	0.44262
40 To 50	9.	36.	0.14754	0.59016
50 To 60	2.	38.	0.03279	0.62295
60 To 70	7.	45.	0.11475	0.7377
70 To 80	4.	49.	0.06557	0.80328
80 To 90	7.	56.	0.11475	0.91803
90 To 100	5.	61.	0.08197	1.
More	0.E+0	61.	0.E+0	1.



Variable #1 (High (>8-12 J/cm2))

Count	61	*Skewness*	0.10008
Mean	48.12296	*Skewness Standard Error*	0.30121
Mean LCL	39.41143	*Kurtosis*	1.99721
Mean UCL	56.8345	*Kurtosis Standard Error*	0.57398
Variance	810.36422	*Alternative Skewness (Fisher's)*	0.10262
Standard Deviation	28.4669	*Alternative Kurtosis (Fisher's)*	-0.98491
Mean Standard Error	3.64481	*Coefficient of Variation*	0.59154
Minimum	0.E+0	*Mean Deviation*	24.02974
Maximum	100.	*Second Moment*	797.07956
Range	100.	*Third Moment*	2,252.21569
Sum	2,935.50078	*Fourth Moment*	1,268,900.38775
Sum Standard Error	222.33357	*Median*	42.85714
Total Sum Squares	189,886.84994	*Median Error*	0.58488
Adjusted Sum Squares	48,621.85309	*Percentile 25% (Q1)*	29.09127
Geometric Mean	33.93624	*Percentile 75% (Q2)*	74.90079
Harmonic Mean	35.88727	*IQR*	45.80953
Mode	0.E+0	*MAD*	22.25914

90-100% Outliers stripped

Alpha value (for confidence interval) 0.02

Variable #1 (50)

Count	55	*Skewness*	0.04658
Mean	43.68486	*Skewness Standard Error*	0.31584
Mean LCL	35.3671	*Kurtosis*	1.96519
Mean UCL	52.00262	*Kurtosis Standard Error*	0.59875
Variance	662.05036	*Alternative Skewness (Fisher's)*	0.04789
Standard Deviation	25.73034	*Alternative Kurtosis (Fisher's)*	-1.01788
Mean Standard Error	3.46948	*Coefficient of Variation*	0.589
Minimum	0.E+0	*Mean Deviation*	21.26355
Maximum	89.87342	*Second Moment*	650.01308
Range	89.87342	*Third Moment*	771.89462
Sum	2,402.66729	*Fourth Moment*	830,326.0237
Sum Standard Error	190.8213	*Median*	41.77215
Total Sum Squares	140,710.9031	*Median Error*	0.58633
Adjusted Sum Squares	35,750.71932	*Percentile 25% (Q1)*	27.67857
Geometric Mean	30.64343	*Percentile 75% (Q2)*	66.66667
Harmonic Mean	33.78517	*IQR*	38.9881
Mode	0.E+0	*MAD*	21.22785

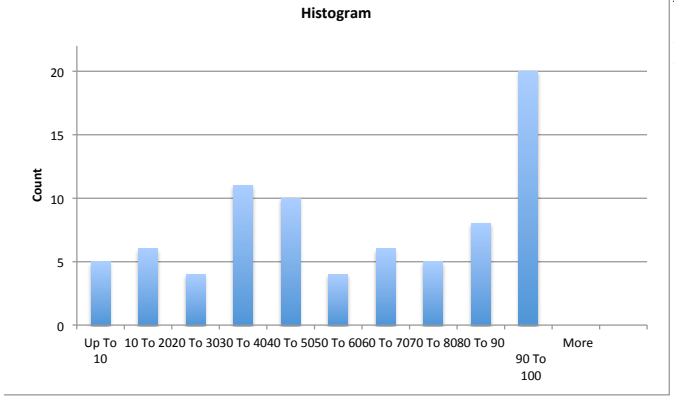
Histogram

No# of valid cases	79

Results for layer #1

Frequency distribution of 1 Tx Only

1 Tx Only	*Count*	*Cumulative Cc*	*Percent*	*Cumulative Percent*
Up To 10	5.	5.	0.06329	0.06329
10 To 20	6.	11.	0.07595	0.13924
20 To 30	4.	15.	0.05063	0.18987
30 To 40	11.	26.	0.13924	0.32911
40 To 50	10.	36.	0.12658	0.4557
50 To 60	4.	40.	0.05063	0.50633
60 To 70	6.	46.	0.07595	0.58228
70 To 80	5.	51.	0.06329	0.64557
80 To 90	8.	59.	0.10127	0.74684
90 To 100	20.	79.	0.25316	1.
More	0.E+0	79.	0.E+0	1.



Alpha value (for confidence interval) 0.02

Variable #1 (1 Tx Only)

Count	79	*Skewness*	-0.25327
Mean	58.45818	*Skewness Standard Error*	0.2671
Mean LCL	50.07302	*Kurtosis*	1.84705
Mean UCL	66.84335	*Kurtosis Standard Error*	0.51455
Variance	984.6514	*Alternative Skewness (Fisher's)*	-0.25819
Standard Deviation	31.37916	*Alternative Kurtosis (Fisher's)*	-1.14942
Mean Standard Error	3.53043	*Coefficient of Variation*	0.53678
Minimum	0.E+0	*Mean Deviation*	27.49052
Maximum	100.	*Second Moment*	972.18745
Range	100.	*Third Moment*	-7,677.15647
Sum	4,618.19641	*Fourth Moment*	1,745,736.33857
Sum Standard Error	278.90403	*Median*	59.52381
Total Sum Squares	346,774.1769	*Median Error*	0.49782
Adjusted Sum Squares	76,802.80888	*Percentile 25% (Q1)*	36.77062
Geometric Mean	42.0519	*Percentile 75% (Q2)*	90.34259
Harmonic Mean	46.75536	*IQR*	53.57197
Mode	100.	*MAD*	27.97619

Sensitivity Test

Stripping 90% outliers

Alpha value (for confidence interval) 0.02

Variable #1 (1 Tx Only)

Count	59	*Skewness*	-0.06226
Mean	45.83176	*Skewness Standard Error*	0.30586
Mean LCL	37.71856	*Kurtosis*	2.08095
Mean UCL	53.94496	*Kurtosis Standard Error*	0.5819
Variance	678.54173	*Alternative Skewness (Fisher's)*	-0.0639
Standard Deviation	26.04883	*Alternative Kurtosis (Fisher's)*	-0.89295
Mean Standard Error	3.39127	*Coefficient of Variation*	0.56836
Minimum	0.E+0	*Mean Deviation*	21.43617
Maximum	89.87342	*Second Moment*	667.04102
Range	89.87342	*Third Moment*	-1,072.5895
Sum	2,704.07377	*Fourth Moment*	925,905.93927
Sum Standard Error	200.08489	*Median*	42.85714
Total Sum Squares	163,287.87716	*Median Error*	0.55335
Adjusted Sum Squares	39,355.42008	*Percentile 25% (Q1)*	30.75457
Geometric Mean	31.82983	*Percentile 75% (Q2)*	67.5
Harmonic Mean	39.85577	*IQR*	36.74543
Mode	0.E+0	*MAD*	20.87912

Histogram

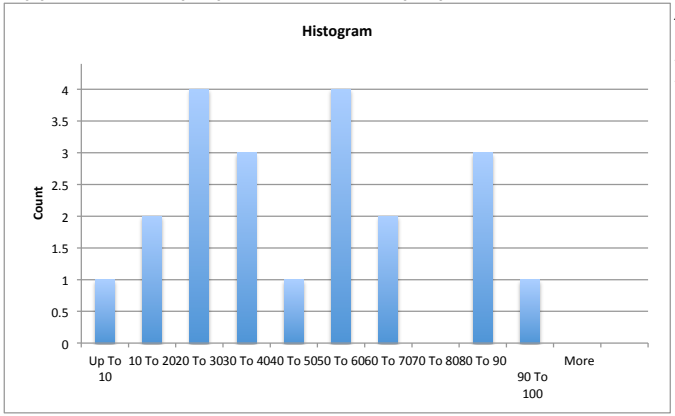
No# of valid cases	21

Alpha value (for confidence interval) 0.02

Results for layer #1

Frequency distribution of 2-3 Tx

2-3 Tx	*Count*	*Cumulative Cc*	*Percent*	*Cumulative Percent*
Up To 10	1.	1.	0.04762	0.04762
10 To 20	2.	3.	0.09524	0.14286
20 To 30	4.	7.	0.19048	0.33333
30 To 40	3.	10.	0.14286	0.47619
40 To 50	1.	11.	0.04762	0.52381
50 To 60	4.	15.	0.19048	0.71429
60 To 70	2.	17.	0.09524	0.80952
70 To 80	0.E+0	17.	0.E+0	0.80952
80 To 90	3.	20.	0.14286	0.95238
90 To 100	1.	21.	0.04762	1.
More	0.E+0	21.	0.E+0	1.



Variable #1 (2-3 Tx)

Count	21	*Skewness*	0.34363
Mean	47.78834	*Skewness Standard Error*	0.47673
Mean LCL	33.24818	*Kurtosis*	2.18216
Mean UCL	62.3285	*Kurtosis Standard Error*	0.83101
Variance	694.72252	*Alternative Skewness (Fisher's*	0.37065
Standard Deviation	26.35759	*Alternative Kurtosis (Fisher's)*	-0.70132
Mean Standard Error	5.7517	*Coefficient of Variation*	0.55155
Minimum	5.	*Mean Deviation*	21.85193
Maximum	100.	*Second Moment*	661.64049
Range	95.	*Third Moment*	5,848.25391
Sum	1,003.55517	*Fourth Moment*	955,278.5762
Sum Standard Error	120.78565	*Median*	46.06742
Total Sum Squares	61,852.68741	*Median Error*	1.57306
Adjusted Sum Squares	13,894.45036	*Percentile 25% (Q1)*	28.92857
Geometric Mean	39.38279	*Percentile 75% (Q2)*	64.47581
Harmonic Mean	28.68315	*IQR*	35.54724
Mode	#N/A	*MAD*	17.49599

Stripping 90% outlier

Alpha value (for confidence interval) 0.02

Variable #1 (5)

Count	19	*Skewness*	0.35151
Mean	47.29238	*Skewness Standard Error*	0.49543
Mean LCL	33.95901	*Kurtosis*	2.05219
Mean UCL	60.62575	*Kurtosis Standard Error*	0.85149
Variance	518.49318	*Alternative Skewness (Fisher's*	0.38239
Standard Deviation	22.77045	*Alternative Kurtosis (Fisher's)*	-0.8574
Mean Standard Error	5.2239	*Coefficient of Variation*	0.48148
Minimum	14.44444	*Mean Deviation*	19.12603
Maximum	89.87342	*Second Moment*	491.20407
Range	75.42897	*Third Moment*	3,826.77325
Sum	898.55517	*Fourth Moment*	495,155.10179
Sum Standard Error	99.25407	*Median*	46.06742
Total Sum Squares	51,827.68741	*Median Error*	1.50203
Adjusted Sum Squares	9,332.87728	*Percentile 25% (Q1)*	29.64286
Geometric Mean	41.80059	*Percentile 75% (Q2)*	63.42742
Harmonic Mean	36.38891	*IQR*	33.78456
Mode	#N/A	*MAD*	16.83581

LIGHT AGE
ADVANCED LASER SYSTEMS

WORKS

Lasers in Onychomycosis

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Lasers in Onychomycosis

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AKG is the owner of Mediprobe Research Inc. He has been a clinical trials investigator for Valeant Canada, Bristol Meyers Squibb, Eli Lilly, Merck, Novartis, Janssen and Allergan. AKG has served as a speaker for Valeant Canada and Bayer.

FCS is an employee of Mediprobe Research Inc.

DFH is CEO of Light Age, Inc.

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Abstract:

Laser systems are an emerging device-based therapy for onychomycosis. To date, reported clinical efficacies, as well as anecdotal clinical results, have varied greatly, and the specific mechanism of action has not been well-elucidated. Here, we provide a distillation of reported data and provide an overview of the mechanisms of action involved together with discussion of how these are impacted by various laser properties. This provides a clearer view of why clinical results have been so diverse and what is needed for more effective laser therapies.

Capsule Summary:

- Laser therapy is a new treatment option for onychomycosis.
- This article describes the specific parameters that must be optimized for effective laser treatment of onychomycosis .
- This article lays a blueprint for future optimization of laser therapies for onychomycosis and helps practitioners to evaluate the parameters of existing laser systems.

Introduction

Onychomycosis is a fungal infection of the finger or toenails.[1] It comprises roughly 50% of all nail disorders and has a global prevalence between 2 and 8%.[2] The primary pathogens are 80-90% dermatophytes, 2-10% yeasts and 2-11% non-dermatophyte molds.[3–11] Pharmacotherapy in onychomycosis is often unsuccessful, with 20-25% rate of relapse/recurrence.[12,13] Older adults are at a higher risk of onychomycosis due to reduced peripheral circulation, brittle nails, and other predisposing health conditions.[13] Onychomycosis has frequent comorbidity with diabetes[14], HIV[15], immunosupression[16], peripheral vascular disease, and smoking[17]. This is problematic because these patients typically require pharmacotherapy for their primary disorder with regimens that may contraindicate use of current anti-fungal systemic drugs.[18,19]

The "gold standard" for onychomycosis therapy has been oral antifungal therapy.[20,21] This can be problematic, as allylamine and azole compounds can have substantial drug and hepatic interactions, which preclude individuals with comorbidities such as heart disease, diabetes or renal dysfunction and may be contraindicated in the case of early recurrence.[20–23] Topical antifungals are preferred in individuals who cannot, or choose not to, take oral antifungals. The two original topical drugs, ciclopirox and amorolfine have limited efficacy.[24,25] New topicals such as efinaconazole and tavaborole may have improved efficacy, but are new molecules that are not yet available for widespread use.[26,27] Topical antifungals require extended periods of administration and their efficacy depends upon patient compliance.

Laser therapy for onychomycosis is an alternative solution to the issues that present with pharmacotherapy. Laser therapy uses light-energy to cause physiological effects, with limited potential for adverse events. Ideally, laser therapy would be a clinic-based procedure without requirement for patient compliance at home. The use of lasers in onychomycosis is a new and developing field, as the

parameters for optimal laser therapy regimens are still being examined. Here, the mechanism of action for lasers in onychomycosis is discussed, along with an examination of the important device parameters involved in achieving a cure.

Mechanism of Action

Lasers emit narrow-spectra light to achieve photo-effects in targeted materials. These effects can induce photochemical, photomechanical and photothermal change in the target. As history has shown, laser-tissue interactions can be safe and effective, however, these sometimes have energetic requirements that exceed the safety parameters approved for clinical use in patients. The primary mechanism of action for most approved medical laser therapies is photothermal, where light energy is converted into heat in the absorbing tissue. Selective photothermolysis is the specific targeting of certain tissue or foreign matter, causing locally confined heating with the intention of causing minimal effects in the surrounding tissue.[28] Usually this is accomplished by matching the wavelength of light to some target-specific chromophore, but other factors are important as well (see below). The goal in treating onychomycosis by thermal means has been to induce high temperatures in the fungal matter under the nail plate for long enough periods to cause fungal thermolysis; this, while keeping the surrounding tissue temperature below the threshold for pain and necrosis (≈45°C).[29,30]

Laser Selectivity

To obtain a selective photothermolytic effect, there are several laser parameters that must be calibrated to selectively target fungal matter. These parameters include the wavelength, spatial and temporal pulse format, peak and average power, pulse energy, and spot size of the laser beam.[31] These parameters need to be designed to support the rapid accumulation and confinement of heat in the fungi, while keeping the temperature in the surrounding nail plate and nail bed low.

Wavelength

The wavelength of the light is a primary laser parameter required for successful selective photothermolysis. The choice of wavelength is dictated by the choice and distribution of chromophores and the required penetration depth of the light. Photo-selectivity is based on the differential spectral characteristics of the target and its surroundings. Ideally, the target will have a strong absorption peak at a wavelength where the surrounding tissue absorbs weakly. However, if this is not possible, selective photothermolysis can be effective if the density of chromophores is much higher in the target than in its surroundings, so more heat is initially imparted into the target than into its surroundings. In dermatological applications, a penetration depth of several millimeters or more is typically required; wavelengths in the deep red or near infrared (750 -1300 nm) have been approved by the FDA for indications requiring deep penetration in all Fitzpatrick skin types.[32] For treatment of onychomycosis, the targeted chromophore should be abundant in fungal hyphae and conidia and less prevalent in the nail plate, nail bed and surrounding skin. Possible chromophores include chitin, melanin and other common fungal pigments. Differential absorption characteristics are commonly believed to be the singular consideration for selective photothermolysis; however, selectivity can be achieved even when the absorption characteristics disfavor the target relative to its surroundings by optimizing the other important laser parameters.

Temporal Pulse Format

The temporal pulse format of the laser refers to the way the pulse energy is distributed in time, including pulse duration, substructure, and pulse repetition rate. For photothermal interaction to be selective it is commonly believed that the duration of the pulse must be shorter than the "thermal relaxation time" of the target. For a given target the thermal relaxation time is related to the target's shape, size, and thermal diffusivity. [28] Fungal hyphae are cylindrical structures with a chitin cell wall, which is a better insulator than the dermal cell membrane. For a roughly cylindrical target having a diameter d and a diffusivity κ, the thermal relation time is roughly $\tau_r \approx d^2/16\kappa$., where κ is the ratio of the thermal conductivity to the volumetric heat capacity. Conidia are roughly spherical, and they have a denser cell wall than hyphae. Since heat loss occurs through the surface, in the typical "optically thin" case where absorption is volumetric, cylindrical hyphae would have a thermal relaxation time roughly 3/4 that of conidia of similar composition and dimension, but the relaxation time for long cylindrical mycelia would be only 2/3 that of the conidia (Figure 1). For concerted thermal confinement (for both fungal structures) the pulse duration should be on a timescale on the order of a few microseconds or shorter.

The temporal pulse format is also critical to selectivity, as proper spacing of pulse components permits dissipation of heat in healthy tissue. A proper choice of temporal format can promote large temperature differences between the target and its surroundings. Dermal cells have a more heat conductive cell membrane and higher water content than fungi, so they have a higher heat capacity and higher thermal conductivity than fungal cells. Ultimately, circulatory and lymphatic systems, as well as convective airflow (if any) and radiation, act to dissipate heat from the irradiated volume. Leveraging on this, an appropriate temporal pulse structure can be designed to keep dermal temperatures low, while maintaining heat confinement and promoting temperature increase in the fungal cells. For selective

fungalysis, effective temporal pulse formats contain pulse components shorter than thermal relaxation times, but with spacings long enough to permit heat dissipation from the dermis, yet not from the fungi.

Microsecond temporal pulse formats having nanosecond components take advantage of the disparities in absorbance, heat capacity and thermal conductivity between the fungi and dermal tissue, as outlined above. Low absorbance of the dermal tissue reduces initial temperature increase and initial volumetric tissue heating. This produces a significant, but nonlethal and typically non-painful, temperature increase in the higher heat capacity dermal tissue. Heat absorbed by the dermal tissue is relatively quickly, dissipated due to its relatively high thermal conductivity and coupling into the circulatory system. Such is not the case for the drier, uncoupled fungi. With proper temporal pulse component spacing, as the next pulse components strike, the internal temperature of the fungal organism increases cumulatively, while the surrounding dermal tissue remains close to its baseline temperature. With optimized pulse structure, typically a pulse having pulse components with sub-microsecond durations and inter-component spacings of many microseconds, the fungal temperature increases in time beyond a fungicidal level, while the temperature of the surrounding tissue increases only marginally. In this way, high internal fungal temperatures can be generated for sufficient time to be lethal, without causing significant pain or damage to the surrounding tissue. Figure 2 illustrates this effect for the fungal “Target” and surrounding (“anti-targeted”) tissue temperatures.

Spatial Format

Treatment consistency, penetration depth, irradiated tissue volume, and reduction of side effects all depend upon the spatial beam format. Spatially uniform beams that have no significant "hot spots" provide the most consistent treatments with reduced side effect. The effective penetration depth and the irradiated tissue volume both depends upon both spot size and beam shape. As light propagates through tissue it tends to disperse radially; as a consequence, the energy that falls on any given area (the "fluence" or light dose) reduces with depth. The rate fluence falls off with penetration depth can be reduced by using larger beam (spot) sizes; however, large, high energy beams heat large tissue volumes, and this can cause significant temperature rise for long enough to cause accompanying side effects. It is best to select a spot size (spot diameter) that provides the fluence needed to treat the fungal pathogen effectively. Typically, for deep red and near IR wavelengths, the spot size optimizes at roughly twice the required penetration depth, so optimal spot sizes tend to be in the few millimeter range. Larger spot sizes can still be effective, as long as the side effects remain tolerable.

Power and Energy Fluence

For a given pulse duration and spot size, the pulse energy of the laser determines the peak power (energy per unit time: W=J/s) and the applied treatment fluence (energy per unit area: J/cm^2). Fluence has become a conventionally recognized measure of light dosage applied to tissue. While the fluence is independent of wavelength and temporal pulse format, it should not be forgotten that all of these parameters are important. The fluence level required for successful photothermolysis varies based on tissue and target properties and on the spatial and temporal pulse formats. For example, the fluence level required for effective treatment can exceed 225 J/cm^2 for longer pulsed systems, but can drop considerably for short pulse durations.[31] This dependence suggests that the mechanism of action may not be solely photothermal, particularly since the effect seems to continue, even when the pulse durations are substantially shorter than the nominal thermal relaxation times of the fungal hyphae and conidia. Typically, fungal thermal relaxation times are on the order of several microseconds, and dermal cell relaxation times are generally shorter.

Many factors affect the choice of the optimal laser system for a given application. For any light-based procedure, efficacy depends upon wavelength, available pulse energy, temporal and spatial pulse format, and pulse rate. Successful photothermolysis for onychomycosis requires that peak power is kept below the ablation threshold of the nail plate and healthy dermal tissues and that the average power (pulse rate, for a given pulse energy: also in given in units of W) is kept sufficiently low to avoid significant volumetric heating of adjacent tissues. The laser parameters, such as pulse repetition rate and spot size can and should be adjusted within the capabilities of the laser system to provide effective and comfortable treatment for both patient and practitioner.

In Vitro Studies of Commercial Laser Models

In vitro studies using commercially available lasers and IPLs have yielded poor to mixed results. Vural *et al*. tested a wide variety of light based devices including intense pulsed light, a 585nm pulse dye laser, a 532 and 1064nm Q-switched laser, a 2940nm Er:YAG laser and a 532nm KTP laser.[33] Only the Q-switched 532nm and 1064nm laser showed growth inhibition of *T. rubrum* (10Hz, 2mm spot, 1-10 J/cm^2). The 1064nm wavelength was most effective at reducing growth rates at fluences of 4 and 8 J/cm^2; the 532nm wavelength was most effective at 8 J/cm^2. Paasch et al. showed minor growth inhibition in *T. interdigitale*, but increased growth for *M. gypseum* at all fluences and no reduction in *T. rubrum* growth at 100J/cm^2. [34] Carney et al. established that heat treatment for *T. rubrum* was temporarily inhibiting at 50°C for 5 minutes and fungicial after 15 minutes.[35] Heat was fungicidal for *E. floccosum* at 10 minutes and inhibiting at 2 minutes. *S. dimidatum* showed reduced growth at 55°C for 5 minutes. Laser irradiation by a 1064nm Nd:YAG laser (LaserGenesis, Cutera) did not result in inhibition of *T. rubrum* at any parameters assayed despite temperatures meeting 40°C.

A mode-locked femtosecond pulsed Ti:Sapphire laser tuned to 800nm was also used in an *in vitro* study on infected nail clippings.[36] *T. rubrum* infections were confirmed by culture (n=99). The nails were irradiated with $7x10^{31}$ photons/m^2-s emitted by 200 fs pulses ($1.4x10^{-4}$ J/cm^2) at a pulse rate of 76MHz (100 W/cm^2) through a variety of apertures. This treatment was completely fungicidal.

Clinical Trials of Commercial Laser Models

Clinical trials have been conducted for a number of "normal-mode" (100 microsecond to 35 millisecond pulse duration), commercial 1064nm Nd:YAG laser models (Table 1). These trials have largely been single-assignment, open-label trials with mixed results ranging from 0-100% mycological cure rates. These trials have varied significantly by pulse format, number and frequency of treatments, and follow-up period. In the single (N=22) randomized, controlled clinical trial, the mycological cure rate did not differ significantly between the treated and control groups.[37] Further randomized, controlled trials are needed to establish efficacy for "normal-mode" 1064nm lasers in treating onychomycosis.

Clinical trials have also been conducted for a Q-switched 1064nm Nd:YAG system having a temporal pulse format containing ns and μs components. The first study was included in the FDA submission and reported significant decrease in dystrophic nail plate area in 95% of participants (Table 2).[38] A second independent study conducted in Europe, used mycology as an inclusion criteria and reported both mycological and clinical outcome. A mycological cure rate in 95.42% of subjects at 3 months was reported, together with high rates of clear nail regrowth.[39] A third study, conducted in Mexico, used the same laser system and claimed similar efficacy.[40] The increased success observed with this Q-switched laser type may be attributable to its particular temporal pulse format.

Conclusions

Laser therapy may be an effective treatment for onychomycosis, but it is a technology that is still in its infancy. The development of lasers for onychomycosis is at an important phase, as primary research on fungal chromophores, the thermal properties of fungal hyphae and a clear understanding of laser penetrance through an infected nail plate are crucial to the achievement of effective selective photothermolysis. The results of early *in vitro* and *in vivo* studies of lasers in onychomycosis have yielded generally poor results.[33–36,41] This is most likely attributable to the use of non-optimal, pre-existing laser systems being applied for a new indication without re-optimization for a fungal target. The FDA clearance of these devices for onychomycosis states that they are substantially equivalent to predicate devices and are indicated for the cosmetic purpose of "Temporary increase of clear nail in patients with onychomycosis". Mycology and clinical response to date indicate that temporal pulse formats having components substantially less than 1µs in duration and operating in the deep red and near infrared regions of the spectrum can be effective in clearing dermatophytic infections below the nail plate, while very long pulses that volumetrically heat are much less effective (Tables 1 and 2).[35,37–39,42–51] We note that heating fungal colonies to sub-lethal temperatures can increase sporation, incubate spores, and increase colony growth rates.

Further study of the optical parameters of dermatophytes and the nail plate will provide more detailed information for the optimization of laser systems for onychomycosis. This should include randomized, clinical trials to determine the efficacy of lasers in clinical populations with onychomycosis. Not all lasers, not even all Nd:YAG lasers, are alike. The wide variation in clinical and mycological results reported to date is likely due to the lack of recognition of the significance of varying laser pulse properties (spatial and temporal) in addition to the effects of wavelength and fluence on the attendant chromophores and mechanisms of action. Better studies controlling for these factors, and the treatment

procedures used, should provide more consistent data and clearer recognition of effective laser treatment parameters and treatment protocols. Present clinical studies, in addition to the large and growing anecdotal base of practitioner treatments, do indicate that appropriate laser therapy can be an important tool in the battle against fungal disease, one of our oldest and most tenacious afflictions.

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Figure Legends:

Figure 1: Dermatophyte hyphae and conidia: Dimensions of heat transfer from fungal structures in onychomycosis.

Abbreviations and Acronyms

cm - centimeter

Er:YAG – erbium yttrium garnet

FDA – United States Food and Drug Administration

J - Joules

Nd:YAG – neodymium yttrium garnet

Nm - nanometers

s - seconds

Ti:Sapphire – titanium sapphire

W - Watts

Table 1: Clinical Trials with Short Pulse Nd:YAG 1064nm Lasers

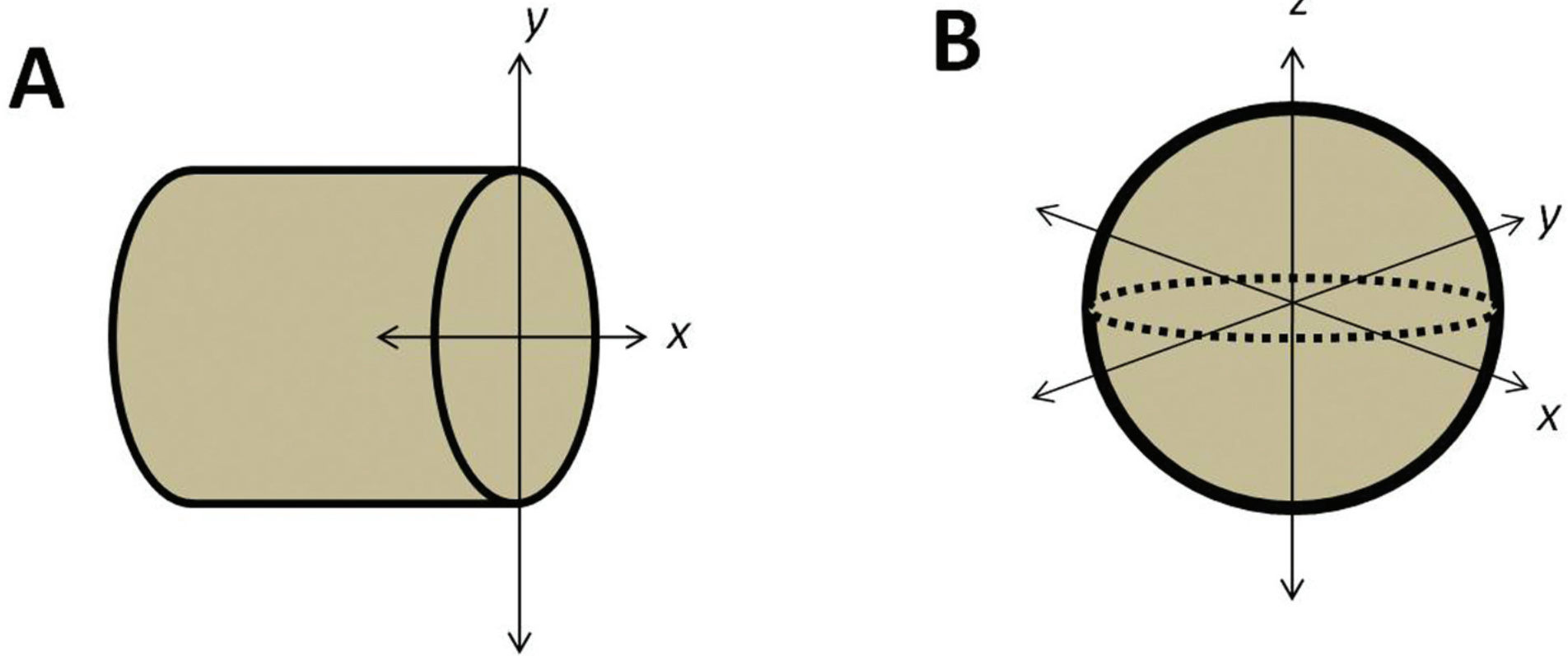
Study	Laser System	Participants	Diagnosis	Pulse duration	Frequency (Hz)	Spot Size (mm)	Energy Fluence (J/cm^2)	# of Tx	Interval	Follow Up Period	MCR (%)	LNG (%)
Harris et al., 2009	PinPointe FootLaser	14	-	-	-	2.5	-	1	1	6 months	-	80%
Kozarev, 2010, 2011	Fotona Dualis SP	162	Culture and KOH	35ms	1	4	35-40	4	1 week	+12 months	100%	-
Weiss, 2011	Cutera GenesisPlus	7	Not specified	300µs	2	5	16	2	6 weeks	12 months	-	70%
Hochman, 2011	Aerolase, LightPod Neo	8	Culture or PAS	650µs	-	2	223	3	3 weeks	4-6 months	87.5%	-
Kimura et al, 2012	Cutera GenesisPlus	13*	KOH	300µs	5	5	14	1-3	4 or 8 weeks	16 weeks	51% *	81% *
Waibel, 2013	Joule ClearSense, Sciton	7	Culture or PAS	300µs	6	-	13	4	1 week	6 months	100%	-
Carney et al, 2014	Laser Genesis, Cutera	14	Culture	300µs	2	5	16	1	-	24 weeks	29%	-
Hollmig et al, 2014	ClearSense, Sciton	27 Laser: 17 Control: 10	Culture or PAS for NDM	300µs	6	6	5	2	2 weeks	3 months	Laser: 33% Control:20%	-
Noguchi et al, 2014	GentleYAG, Candela	12	Culture	500µs	2	6	10	3	4 weeks	6 months	0%	-
Moon et al, 2014	ClearSense, Sciton	13 (43 nails)	Culture and KOH	300µs	5	6	5	5	4 weeks	6 months	70%*	-
Hees et al, 2014	Elite, Cynosure	10	Culture and KOH	40ms 100µs	-	3	50	2	4 weeks	9 months	20%	-
	PinPointe Footlaser				-	1.5	25.5				20%	-

*-reported as nails not participants., MCR – mycological cure rate, LNG – increase in clear linear nail growth >2mm

Table 2: Clinical Trials with Q-switched Nd:YAG 1064nm Lasers

Study	Laser System	Participants	Diagnosis	Pulse duration	Frequency (Hz)	Spot Size (mm)	Energy Fluence (J/cm^2)	# of Tx	Interval	Follow Up Period	MCR (%)	CCR	LNG (%)
510(k) K110370, 2011	Q-Clear Light Age,	100	-	ns, µs	1	2.5-6	14	1	-	-	-		95%
Kalokasidis et al. 2014	Q-Clear Light Age	100	Culture	ns, µs	5	2.5	14	2	30 days	3 months	95.42%		96.7%
Garcia Galvan et al. 2014	Q-Clear, Light Age	62	KOH	ns, µs	3	3	19	1	-	9	100%	100%	

CCR– clinical cure rate,MCR – mycological cure rate,LNG – increase in clear linear nail growth >2mm or significant reduction in affected nail area.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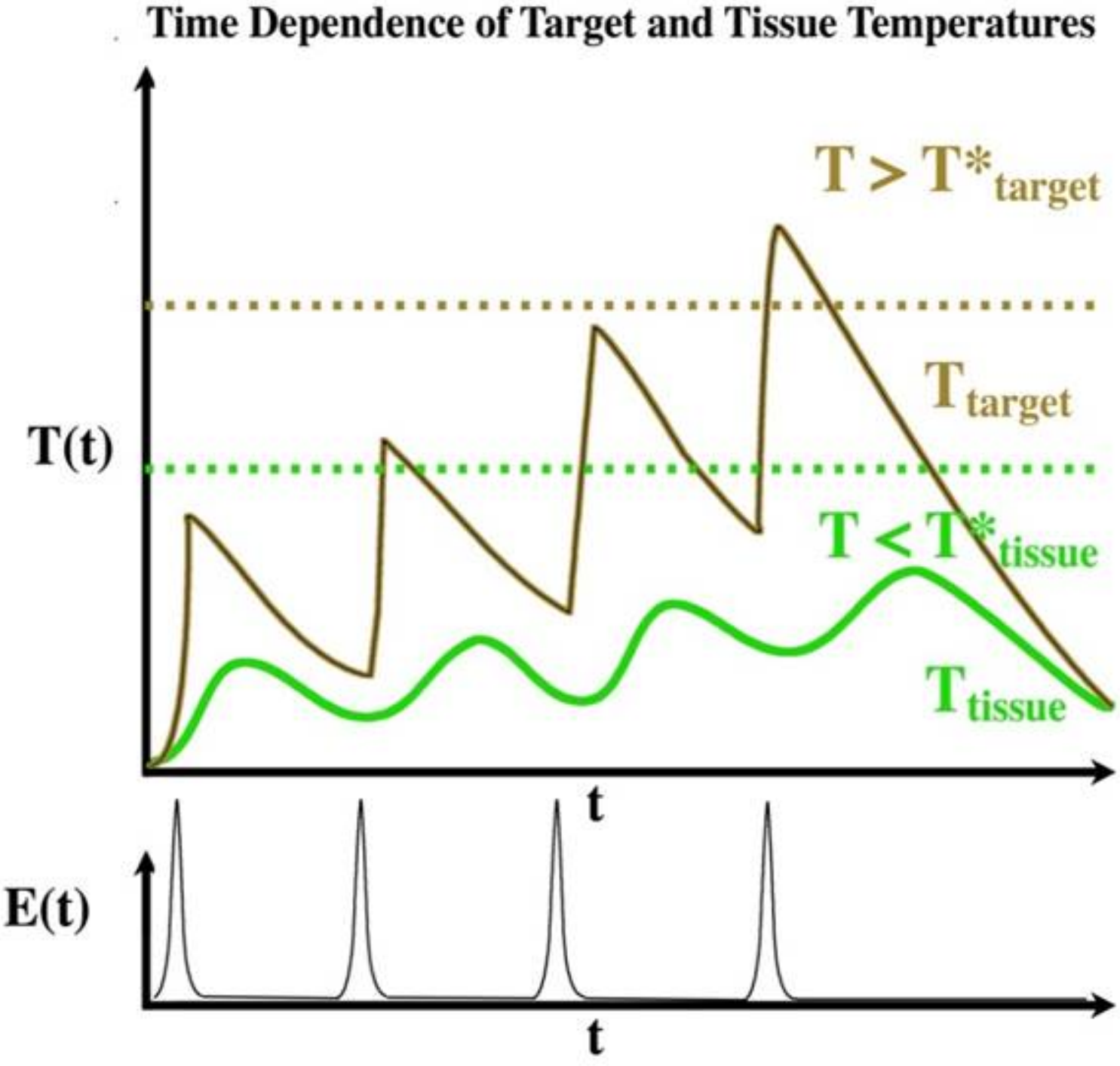

Figure : Schematic dependence of temperature increases in target and anti-targeted tissue as functions of time for a time-structured laser pulse format given by E(t), the time-dependent laser pulse energy. The dotted lines indicate the temperature levels sufficient to damage the target (T^*_{target}) and anti-target (T^*_{tissue}), respectively.

LIGHT AGE
ADVANCED LASER SYSTEMS

Lack of Efficacy with Long Pulse Nd:YAG Lasers for the treatment of Onychomycosis

DO NOT

Lack of efficacy with 1064-nm neodymium:yttrium-aluminum-garnet laser for the treatment of onychomycosis: A randomized, controlled trial

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Background: Laser therapies have been Food and Drug Administration approved for temporary nail plate clearance; however, there is minimal evidence of their long-term efficacy.

Objective: We sought to evaluate the clinical and mycological clearance of toenails treated with 1064-nm neodymium:yttrium-aluminum-garnet laser versus no treatment.

Methods: This was a randomized, controlled, single-center trial comparing 2 treatments with 1064-nm neodymium:yttrium-aluminum-garnet laser (fluence of 5 J/cm^2, rate of 6 Hz) spaced 2 weeks apart versus no treatment in 27 patients (N = 125 affected nails) with clinical and mycological diagnosis of onychomycosis. At 3 months, patients were assessed with mycological cultures and proximal nail plate measurements. Patients treated with laser were also assessed with proximal nail plate measurements at 12 months.

Results: At 3 months, 33% of patients treated with laser achieved a negative mycological culture compared with 20% of the control group (P = .49), and had more proximal nail plate clearance compared with control subjects (0.44 vs 0.15 mm, P = .18), which was not statistically significant. At 12 months, there was no difference in nail plate clearance between laser versus control subjects (0.24 vs 0.15 mm, P = .59).

Limitations: Our study was limited by the small sample size and number of treatments.

Conclusions: There was no significant mycological culture or clinical nail plate clearance with 1064-nm neodymium:yttrium-aluminum-garnet laser compared with control. (J Am Acad Dermatol 2014;70:911-7.)

Key words: 1064-nm neodymium:yttrium-aluminum-garnet laser; onychomycosis.

Onychomycosis is exceedingly common, afflicting approximately 14% of the US population and representing the most common nail disorder in adults.[1] Modalities for treatment of onychomycosis include pharmacologic and mechanical, with a recent focus on laser methods. Treatment selection is often based on the number and location of affected nails, type of causative fungi, concomitant systemic medications, treatment costs, and patient preference.[2] Clinicians are required to weigh both the likelihood and value of eliminating an individual patient's toenail fungus against the likelihood of

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The JOULE ClearSense handpiece was loaned to Stanford Department of Dermatology from Sciton Inc for the purposes of the study.
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recurrence if treatment is successful and possible adverse events associated with systemic antifungal medications.[3,4]

Although the mechanism of action is not clearly understood, lasers have been proposed to penetrate through the nail plate and reach a temperature that kills the colonized fungus. Five lasers are currently Food and Drug Administration (FDA) approved for the temporary increase of clear nail in patients with onychomycosis: PinPointe FootLaser (PinPointe USA Inc, Chico, CA),[5] Cutera GenesisPlus laser system (Cutera Inc, Brisbane, CA),[6] CoolTouch VARIA laser (CoolTouch Inc, Roseville, CA),[7] Light Age Q-Clear laser (Light Age Inc, Somerset, NJ),[8] and Sciton Inc JOULE ClearSense (Sciton Inc, Palo Alto, CA).[9] Four of the 5 lasers use a 1064-nm wavelength and deliver energy in a short pulse duration (microseconds).

CAPSULE SUMMARY

- There are limited data supporting the use of laser therapies for onychomycosis.
- In our study, 1064-nm neodymium:yttrium-aluminum-garnet laser treatment at a fluence of 5 J/cm^2 (rate of 6 Hz) did not achieve negative mycological culture or long-term proximal nail plate clearance.
- The 1064-nm neodymium:yttrium-aluminum-garnet laser using these settings does not appear to be an effective treatment for onychomycosis.

However, there are limited data supporting the use of laser therapies for onychomycosis with the only published clinical trial evaluating the use of a 870- and 930-nm laser,[10,11] which is not FDA approved for treatment of onychomycosis. Of the aforementioned FDA-approved lasers, medical device approval is primarily based on being substantially equivalent to currently marketed devices.[5-9] The cost of 1 treatment session from these lasers ranges from $400 to $1200, yet none have been rigorously studied against a control population or with long-term follow-up. We conducted a randomized, controlled trial of 1064-nm neodymium:yttrium-aluminum-garnet (Nd:YAG) laser for the treatment of onychomycosis.

METHODS

The study was a randomized, controlled trial conducted at a single academic institution. The primary end point for the study was the percentage of patients with a negative mycological culture from all clinically involved nails at 3 months, and the secondary end point was the difference in clinical proximal nail plate clearance at 3 months and at 12 months. An additional secondary end point was the number of nails with complete clinical nail plate clearance at 3 months and, for laser patients, at 12 months. This study was approved by the institutional review board at the Stanford University Medical Center (clinicaltrials.gov identifier: NCT01666002), and all patients signed written informed consent. This study was conducted in accordance with the CONSORT statement.[12]

We enrolled adults (18-75 years) with a clinical diagnosis of onychomycosis from the dermatology clinic at Stanford between July and December 2011. The key inclusion criterion was a diagnosis of onychomycosis by clinical toenail morphology confirmed by positive culture. Patients whose cultures revealed nondermatophyte molds were included if periodic acid—Schiff staining-assisted microscopic evaluation was positive (4 in laser group; 3 in control group). All patients met diagnostic criteria for onychomycosis as defined by prior studies.[13] Patients were not excluded based on the severity of disease or prior treatment regimen. Patients were randomized following simple randomization procedures (computerized random number generator) in a 2:1 ratio into laser or control groups. Both groups underwent evaluation by study dermatologists at baseline and follow-up. Photographs, nail plate measurements, and fungal cultures from all clinically suspicious toenails were obtained at each study visit. We followed up the laser-treated group for an additional 12 months to assess long-term clinical clearance with proximal nail plate measurements. Treatment was performed using the 1064-nm Nd:YAG laser fitted with the 6-mm JOULE ClearSense handpiece (Sciton Inc, Palo Alto, CA). Laser settings were those recommended by the laser manufacturer and included a fluence of 5 J/cm^2, pulse width of 0.3 milliseconds, spot size of 6 mm, and rate of 6 Hz to achieve a measured target temperature of 40°C to 42°C. The entire nail plate, proximal and lateral nailfolds, and matrix in all 10 toenails (regardless of clinical or mycological status) were treated with 2 to 3 passes of the laser. Patients in the laser group underwent 2 treatments separated by 2 weeks. Patients in the control group were not treated and were observed at baseline and 3-month follow-up. All patients randomized to control were offered a single laser treatment at the end of the 3-month observation period. Patients in both groups underwent no other types of treatment (no oral or topical antifungal medications) during the course of the study.

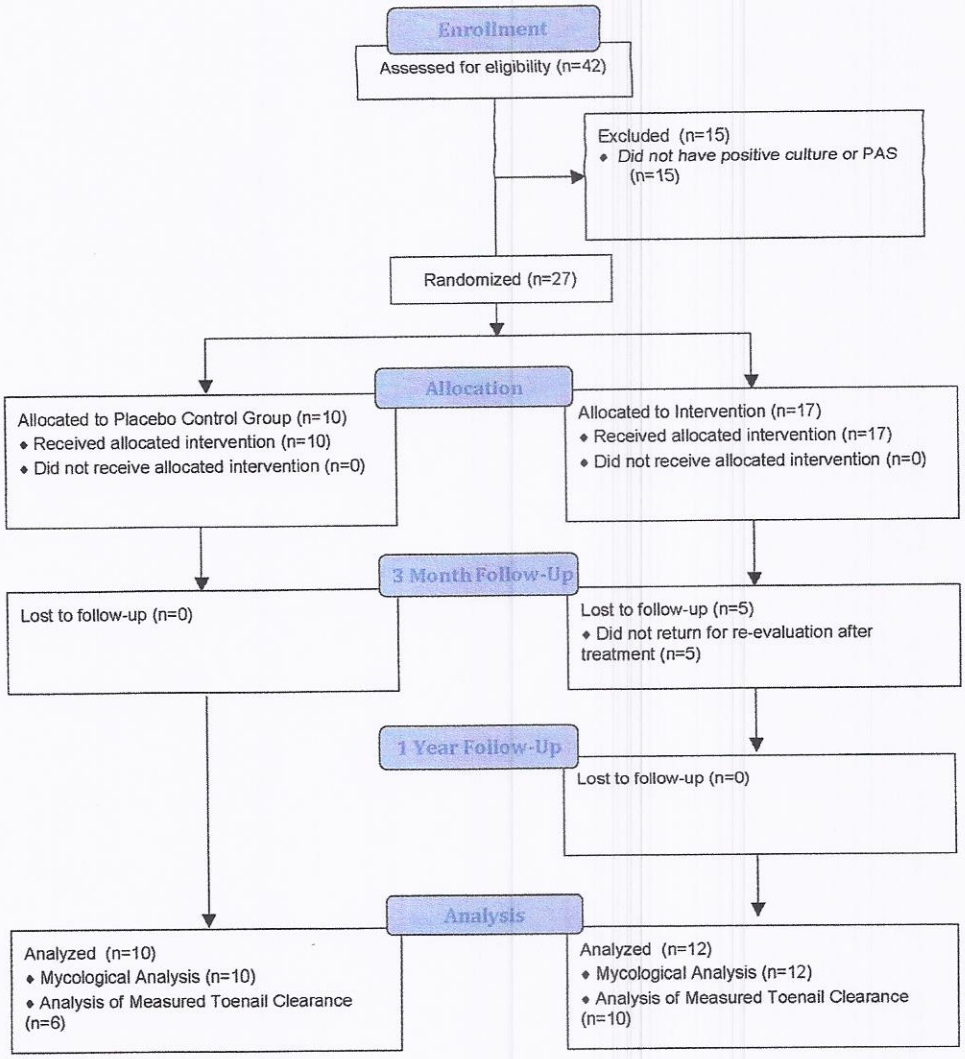

Fig 1. Eligibility flow diagram; 42 patients were assessed and 27 met eligibility criteria of a diagnosis of onychomycosis by clinical toenail morphology confirmed by positive culture. *PAS*, Periodic acid–Schiff.

Sample size calculations (STATA 10, StataCorp, College Station, TX) indicated that we needed to enroll 24 patients at a 2:1 ratio for 80% power to detect a minimal proximal nail plate difference of 2.5 mm in the 2 groups with a 2-sided alpha of 0.05. The primary end point was the percentage of patients with a negative mycological culture. The secondary end point was proximal nail plate clearance as assessed directly by a single study physician, who measured the clinical involvement—defined as total length of abnormal nail per each nail—of each of the patients' toenails, and confirmed by digital analysis of toenail photographs with ImageJ software (Rasband WS, ImageJ, US National Institutes of Health, Bethesda, MD, http://imagej.nih.gov./ij/). Descriptive statistics were provided for demographic and baseline clinical parameters. Continuous variables were compared using nonparametric *t* tests, and percent of response to treatment analyzed using Fisher exact tests.

RESULTS

In all, 42 patients were assessed for eligibility (Fig 1). Fifteen patients were excluded because of lack of positive mycological culture. The remaining 27 patients were randomized in a 2:1 ratio into laser (N = 17) and control (N = 10) groups. Five of the patients in the laser group did not return for follow-up, with 12 of patients in this group completing the study, whereas all of the patients in the control group completed the study. The 12 patients in the laser group exhibited 57 clinically involved toenails, whereas the 10 patients in the control group exhibited 68 clinically involved nails.

Table I. Baseline characteristics

	Laser N = 12 patients (N = 57 nails) Mean (SD)	Control N = 10 patients (N = 68 nails) Mean (SD)	*P* value
Age, y	53 (14)	65 (8)	.03
Male	83%	80%	.86
Interval for 3-mo follow-up, d (SD)	101 (13)	86 (29)	.14
Interval for 12-mo follow-up, d (SD)*	453 (43)		
Clinical involvement per affected nail at baseline, mm†	8.0 (3.9)	7.5 (3.5)	.52
Baseline culture with dermatophyte	67%	70%	.84

*Includes those completing 12-mo clinical assessment visit (N = 10 patients, correlates with 39 nails).
†For nail measurements, 6 control subjects (N = 39) and 10 laser patients (N = 39) were available.

Table I describes the baseline characteristics of the 22 patients who completed the study. Patients in the laser and control groups differed in age (64.9 vs 53.4 years, $P = .03$) but not in gender, interval days for follow-up, extent of clinical nail involvement, or percent with dermatophyte species at baseline culture. Patients randomized to laser treatment received 2 sessions of 1064-nm Nd:YAG at a fluence of 5 J/cm^2, pulse width of 0.3 milliseconds, spot size of 6 mm, and rate of 6 Hz to achieve a measured target temperature of 40°C to 42°C. No patients reported complications or adverse events after 2 sessions.

After 3 months, 4 of 12 patients (33%) in the laser group had negative fungal cultures (Table II). Of the 4 patients in the laser group with baseline cultures positive for a nondermatophyte mold (*Acremonium*, *Aspergillus*, *Cladosporium*), 2 (50%) had negative fungal cultures. After 3 months of observation, 2 of 10 (20%) control subjects had negative cultures. Of the 3 patients in the control group with baseline cultures positive for a nondermatophyte mold (*Aureobasidium*, *Aspergillus*, *Scopulariopsis*), 1 (33%) had a negative fungal culture. There was no significant difference in the percentage of patients with negative nail cultures between laser versus control groups ($P = .49$). Our secondary end point of proximal nail clearance was assessed in a subset of patients (N = 16) at 3 months (Table II). Patients treated with laser had more proximal nail plate clearance compared with control subjects (0.44 vs 0.15 mm, $P = .18$), however, this did not reach statistical significance. After 12 months, the modest improvement of proximal nail plate clearance seen in the laser group was not sustained. At 12 months,

Table II. Clinical and mycological results

	Laser N = 12 patients (N = 57 nails) Mean (SD)	Control N = 10 patients (N = 68 nails) Mean (SD)	*P* value
Negative mycological culture at 3-mo follow-up	33%	20%	.49
3-mo Follow-up			
Proximal nail plate clearance per affected nail, mm*†	0.44 (1.1)	0.15 (0.7)	.18
Nails with complete clinical clearance*	1 Nail	0 Nails	.32
12-mo Follow-up‡			
Proximal nail plate clearance per affected nail, mm*†	0.24 (0.6)		.59
Nails with complete clinical clearance*	1 Nail		.32

*For nail measurements, 6 control subjects (N = 39) and 10 laser patients (N = 39) were available.
†Measures the proximal nail plate clearance as compared with baseline values.
‡Compared with proximal nail plate clearance at 3 mo in control group.

there was no difference in proximal nail plate clearance in patients treated with laser compared with control at baseline (0.24 vs 0.15 mm, $P = .59$) (Fig 2). One nail (2.5% of total treated nails) in the laser group had complete clinical nail plate clearance; however, this was not statistically different from the control group ($P = .32$).

DISCUSSION

We performed a randomized controlled trial of 1064-nm Nd:YAG laser treatment for onychomycosis. We found that 1064-nm Nd:YAG laser treatment did not improve mycological culture or long-term proximal nail plate clearance (Fig 3). Similar to other studies, we detected a trend of improved proximal nail plate clearance at 3 months, but this clearance did not persist at 12 months. Our study supports the recent in vitro findings by Hees et al[14] who treated fungal colony isolates in a Petri dish with the 1064-nm Q-switched Nd:YAG laser and the 532-nm Q-switched Nd:YAG laser and found no effect on fungal growth.

The results from our study contrast with 2 in vitro studies and 2 randomized controlled trials. Nd:YAG lasers reduced fungal growth in vitro after 6 days[15] and reduced fungal growth from treated clipped toenails. In noncontrolled studies, 1064-nm Nd:YAG laser achieved negative fungal cultures in 7 of 8

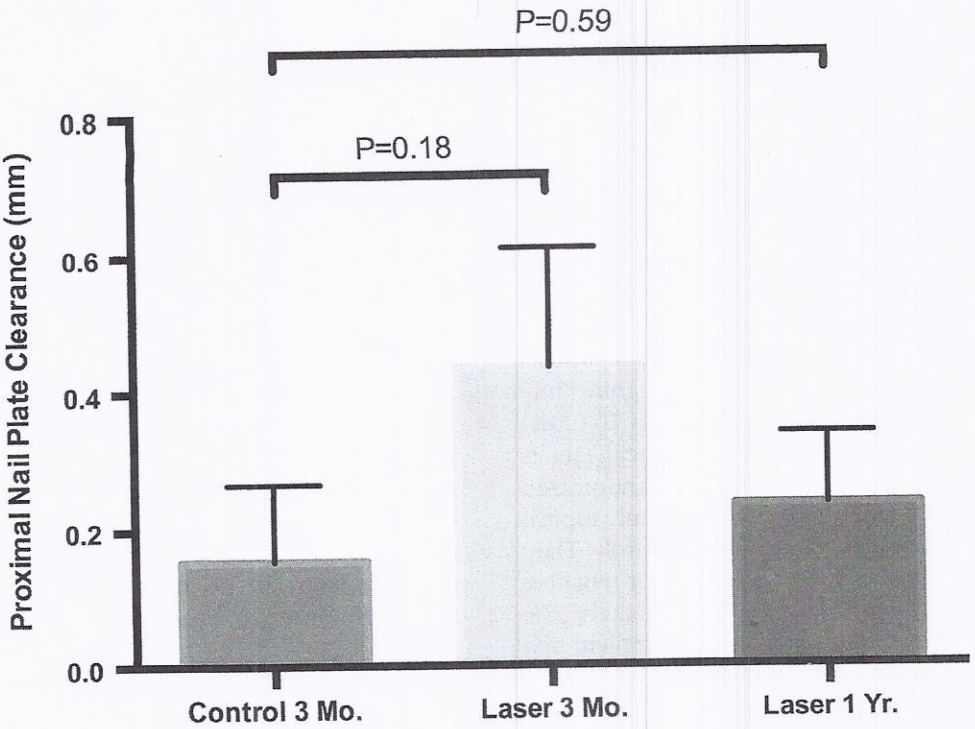

Fig 2. Mean proximal nail plate clearance with SEM and respective *P* values. Patients treated with neodymium:yttrium-aluminum-garnet (Nd:YAG) had more proximal nail plate clearance compared with control subjects (0.44 vs 0.15 mm, $P = .18$), however, this did not reach statistical significance. After 12 months, there was no difference in proximal nail plate clearance in patients treated with Nd:YAG compared with control at baseline (0.24 vs 0.15 mm, $P = .59$).

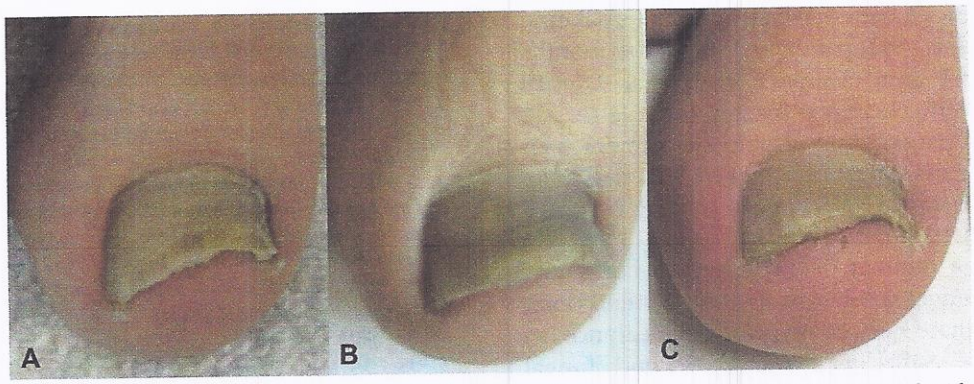

Fig 3. Onychomycosis. Photographs of right first toe from 1 representative patient treated with neodymium:yttrium-aluminum-garnet with no change in proximal nail plate clearance at baseline before intervention (**A**), 3 months (**B**), and 12 months (**C**).

patients after 2 to 3 treatment sessions.[16] and achieved negative potassium hydroxide microscopy and complete clinical nail plate clearance in 51%.[17]

We are aware of only 2 randomized controlled trials, both industry sponsored. Landsman et al[11] randomized 34 patients (N = 37 infected toenails) to no treatment control or 4 treatments with a 870- and 930-nm laser. At 6 months, the treatment group exhibited a 3.5-mm average of clear nail growth compared with 0.4 mm in the control group ($P = .0015$). At 9 months, 38% of the treatment group and 15% of the control group were mycologically clear.[10] Harris et al[18] also found a 3.3-mm average of new clear nail growth after 1 treatment with a 0.45-millisecond pulsed 1064-nm Nd:YAG laser compared with control (N = 15 nails, $P < .001$). The transient nail clearance is likely a result of lasers changing the nail bed environment making it less hospitable for fungal growth.[19] Carney et al[19] showed that fungal wall lysis required temperatures

above 50°C to achieve a fungicidal effect, which is much higher than 42°C achieved during routine clinical application.

Our study allocated patients, rather than individual toenails, to laser and control groups, which we thought allowed for a more clinically meaningful analysis. We treated all 10 toenails and obtained cultures from all clinically suspicious nails. As such, we were able to preclude concerns for reinfection from untreated but subclinically infected toenails.

Our primary end point of negative mycological culture at 3 months was seen in 4 of 12 (33%) patients in the laser group, which did not differ significantly from negative mycological cultures in 2 of 10 (20%) control subjects. This negative mycological rate is similar to that seen in 2 studies that randomized approximately 200 nails to 12 months of daily topical nail lacquer ciclopirox or vehicle control. They found a mycological clearance, defined by negative culture and KOH microscopy, of approximately 32% versus 10% in the control group, and complete nail plate clearance in 5% to 8% of treatment patients compared with 0% to 1% of vehicle control.[20] A recent trial of daily application of Vick's VapoRub (Procter & Gamble, Cincinnati, OH) (N = 18 patients) also found 28% mycological and complete nail plate clearance at 12 months.[21]

The current gold standard for treatment of onychomycosis is systemic antifungal therapy with terbinafine. A meta-analysis of daily terbinafine (N = 18 studies, 993 patients) for 3 to 4 months showed mycological clearance of 76% at 9 to 18 months and 59% clearance for itraconazole (N = 7 studies, 1131 patients). In addition, treatment with terbinafine or itraconazole achieved 70% complete nail plate clearance in contrast to the 2.5% we detected with 1064-nm Nd:YAG.[22]

There are a number of limitations to our study. First, use of fungal cultures as the primary end point may overestimate rates of mycological clearance, as false-negative results are common. Second, fungal cultures were taken from all affected nails, potentially reducing the chance of detecting a mycological difference. However, this was also done for the control group. Third, we did not obtain fungal cultures from patients at 12 months posttreatment because we did not see any clinical improvement from previous visits. Fourth, we did not follow up control subjects at 12 months (because they crossed over) and thus 12-month laser group comparisons were made to control at 3 months. Fifth, this trial was powered to be able to detect only larger differences (at least 2.5 mm) between the 2 groups. This trial was underpowered to detect differences smaller than 2.5 mm; however we chose a 2.5-mm difference as we thought that differences less than 2.5 mm would not be clinically meaningful. In addition, our sample size was not large enough to allow stratification by type of causative organism and severity of involved toenails. Lastly, we may have needed more treatment sessions and cannot rule out clearance with different treatment regimens; however, 2 sessions is the recommended regimen by laser companies and 1 to 2 sessions have been effectively used by many other studies to date.

Our results suggest the 1064-nm Nd:YAG device is not effective for onychomycosis as indicated by a lack of significant mycological clearance at 3 months and a transient trend toward proximal nail clearing that was not sustained at long-term follow-up. Our findings cannot be generalized to other lasers with different wavelengths, fluences, or pulse rates. Future research should further examine the efficacy of other lasers in nonindustry-sponsored trials to fully evaluate any role of lasers for onychomycosis.

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