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Evaluation of a dual-diode 635- and 405-nm low-level laser device for the treatment of distal lateral subungual onychomycosis

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Running head: LLLT at 405- and 635-nm for the treatment of DLSO

Key words: low-level laser therapy, distal lateral subungual onychomycosis, nail clearance.

Summary

Introduction: Onychomycosis has a complex pathogenesis that has limited the development of effective therapies and resulted in a high prevalence of reinfection. One therapeutic approach with promising clinical outcomes to treat distal-lateral subungual onychomycosis (DLSO) has been the application of a dual-diode low-level laser device, emitting at 405 and 635 nm.

Methods: In this prospective study, we qualified and enrolled 105 toes with DLSO. Toes were evaluated on four occasions: baseline and 2, 3, and 6 months post-procedure. The treatment phase consisted of two independent 12-min treatments separated by 7 days. No adjunctive modalities were used. Study success criterion was a minimum improvement of percent nail clarity of $\geq 25.0\%$ at 3 months.

Results: Compared with baseline, statistically significant mean changes in nail clearance of 4.9 mm and 6.15 mm were observed at 3 and 6 months post-procedure, respectively ($df = 104$; $p < 0.0001$). This corresponded to a 30.4% and 36.3% improvement in percent nail clarity at 3 and 6 months, respectively. Sixty-two percent of enrolled toes satisfied the individual study success criterion.

Conclusions: These data and the absence of adverse events demonstrate the efficacy and safety of the dual-diode low-level laser device in the treatment of DLSO.

Introduction

Onychomycosis (OM) is a chronic fungal infection of the nail plate, nail bed, or both, and has been estimated to affect 3-10% of the general population, with the incidence rising sharply, to nearly 30%, in patients over age 60 [1-4]. Although OM is categorized into five different types, distal lateral subungual onychomycosis (DLSO) is the most common clinical presentation, reported to be as high as 90% of OM cases in a 4,096 patient study [4,5]. If left untreated, DLSO can lead to subungual hyperkeratosis, which, in turn, can aggregate substantial keratinous debris and induce both nail pressure and pain [6,7]. Additionally, DLSO has been reported to predispose patients to more serious comorbidities, such as bacterial infection, foot ulceration, cellulitis, thrombophlebitis, and gangrene [7-11]. Successful treatment of DLSO has been hindered by inappropriate therapeutic solutions, with poor clinical outcomes, high rates of recurrence, low patient compliance, or risks of adverse events [7]. Low-level laser therapy (LLLT), however, has emerged as an alternative remedy with demonstrated positive clinical outcomes in the treatment of OM.

LLLT adheres to the tenets of photochemistry, the discipline that studies the effects of light on intracellular secondary reactions [12-15]. The laser's influence on cell behavior follows modulation of the cell's bioenergetics: specifically, upregulation of adenosine triphosphate and reactive oxygen species (ROS) synthesis [12]. This mechanism can be likened to the agonist effect of a drug, which describes the use of a certain molecule to start a secondary cascade. Laser therapy uses photonic energy to modulate secondary cellular reactions. Supported by nearly four decades of clinical research, LLLT has lead to favorable clinical outcomes without serious adverse events.

Regarding the treatment of OM, LLLT, when delivered with specific parameters, has been shown to decrease dermatophyte colonization and to strengthen the function of phagocytes, such as neutrophils and macrophages [16-24]. Accordingly, stimulation of the body's endogenous defense systems and antimicrobial effects suggest LLLT as a potentially suitable treatment for OM. Here, we evaluated patients presenting with three distinct stages of distal-lateral OM to examine the efficacy of a dual-wavelength laser device.

Patients and Methods

A prospective, non-randomized, non-controlled study was conducted from February 2011 to February 2012. Participants who presented with typical clinical patterns of OM of the great toenail were evaluated, qualified, and enrolled in the study. In total, 105 subjects were qualified and enrolled.

All enrolled subjects satisfied the study inclusion criteria: OM in at least one great toenail, disease involvement in the great toenail of at least 25%, subject was willing and able to refrain from the use of nail cosmetics, such as clear and/or colored nail lacquers, throughout study participation, and aged 18 years or older. Patients were excluded if they satisfied any of the following criteria: spikes of disease extending to the nail matrix in the affected great toenail, infection involving the lunula of the affected great toenail, the affected great toenail having less than 2 mm clear (unaffected) nail plate length beyond the proximal fold, the presence of dermatophytoma (defined as thick masses of fungal hyphae and necrotic keratin between the nail plate and nail bed) on the affected great toenail, chronic plantar (moccasin) tinea pedis, a history of current or past psoriasis of the skin and/or nails, concurrent lichen planus, onychogryphosis, other conditions affecting the great toenail (proximal subungual onychomycosis, white

superficial onychomycosis, exclusively lateral disease), confounding problems/abnormalities of the great toenail, trauma to the affected toenail, use of oral antifungal agents in the past 6 months, use of topical antifungal agents in the past 1 month, the subject was unwilling or unable to refrain from using other (non-study) treatments (traditional or alternative) for the toenail throughout the study, cancer and/or treatment for any type of cancer within the last 6 months, peripheral vascular disease or peripheral circulatory impairment, history of uncontrolled diabetes mellitus, pregnant, breast feeding, or planning pregnancy prior to the end of study participation, serious mental illness, or participation in a clinical study or any other type of research in the past 30 days.

Subject Demographics

In total, 105 subjects (64 females, 41 males) were enrolled. The mean subject age was 59.50 (range, 19-89) years. Of all the great toenails treated, 46.7% were right and 53.3% were left. Three categories (< 25%, 26-50%, and 51-75%) were used to categorize subjects according to baseline percent nail clarity. Of the 105 toes, 30, 35, and 40 were categorized as < 25%, 26-50%, and 51-75% clarity, respectively.

Patients were not compensated financially to participate in the clinical study. Furthermore, patients were recruited from the physician's existing patient pool who, at one time, actively sought treatment of OM.

Assessment

Study outcome measures included high-resolution photographs, measurement of change in clear nail bed (mm), and calculation to determine % clearance. Pre-procedure (baseline) images were

taken of the infected nail, which were then compared with procedure and post-procedure images. The study success criterion was a minimum improvement of percent nail clarity of ≥ 25.0%. Additionally, 60% of all treated toes were anticipated to reach the toenail success criterion. Toes were evaluated at four separate time points: baseline and 2, 3, and 6 months post-procedure. Nail assessments were made by the study's principle investigators and measurements and calculated percentages were agreed by consensus.

Device Intervention

Subjects received treatment with a dual-diode low-level laser device that emitted two independent, rotating line-generated coherent beams, each emitting a separate and discrete wavelength (405 nm and 635 nm) with a total output intensity of ~32 mW (the Lunula Laser, Erchonia Corporation).

Treatment Administration

The treatment administration phase consisted of two independent 12-min treatments separated by 7 days. Patients removed any external wear and placed the infected foot into the treatment device. No debridement was performed prior to treatment. All toes of the infected extremity, regardless of clinical presentation of OM, received equal exposure to the emitted laser energy. Following treatment, patients were provided with a new pair of socks and were provided with Tineacide antifungal spray to be applied to shoes and other environments conducive to fungal growth. Patients were instructed not to apply the spray directly to the infected extremity.

Data Analysis

A paired *t*-test was used to assess the independent groups mean change in the measurement of nail clearance. Additionally, a paired *t*-test was used to evaluate the independent groups mean change for the percent nail clearance. A repeated-measures ANOVA was used to assess the four independent sample means.

Results

All study toes (n = 105) were categorized into three groups based on their initial percent nail clarity. Comparison of subject ages within the categories showed no statistically significant difference ($F = 0.015$; $p > 0.05$). Compared with baseline, statistically significant mean changes in nail clearance, of 4.9 mm and 6.15 mm, were observed at 3 and 6 months post-procedure, respectively (Table 1; $df = 104$; $p < 0.0001$).

Table 1

Across each study evaluation point, analyses of the correlated samples showed a statistically significant clearance of the nail plate ($F = 240.3$; $p < 0.0001$). Subsequent Tukey honestly significant difference (HSD) analysis demonstrated a statistically significant change (in mm) between each of the four study evaluation points (Table 2).

Table 2

For each individual baseline category, a statistically significant mean change was observed from baseline at 3 and 6 months (Table 3).

Table 3

Calculation of percent nail clearance at each evaluation point showed the changes in mean percent nail clarity to be statistically significant across the four correlated samples ($F = 199.2$; $p < 0.0001$; Fig. 1).

Figure 1

Compared with the initial baseline clearance of 43.4%, post-procedure evaluation at 3 and 6 months showed nail clarity of 73.8% and 79.8%, respectively (Table 4).

Table 4

For each individual baseline category, when the 6-month percent nail clarity was compared with the baseline percent nail clarity, a statistically significant percent mean change was seen (Table 5).

Table 5

Across each study evaluation point, analysis of the correlated samples showed a statistically significant percent clearance of the nail plate ($F = 199.2$; $p < 0.0001$). Subsequent Tukey HSD analysis demonstrated a statistically significant change in percent nail clearance between each of the four study evaluation points ($p < 0.01$).

The before and after images show the change in percent nail clarity for each baseline category. The first image depicts a toe in the 51-75% category with 100% clarity reported at 6 months post-treatment (Fig. 2). The next images illustrate a toe assigned to the 26-50% baseline category with a 100% clarity reported (Fig. 3). The last image was assigned to the < 25% category with an observed nail clearance of 100% at 6 months post-treatment (Fig. 4).

Figures 2, 3, 4

Of the total enrolled population ($n = 105$), 65 (62%) and 80 (76%) satisfied the individual study success criterion are months 3 and 6, respectively (Table 6).

Table 6

Both evaluation periods exceeded the pre-established goal of 60% by 2% and 16%, respectively. Additionally, ANOVA reported no statistically significant difference when comparing mean change for left or right toenail infection or gender.

Discussion

These data, coupled with the absence of adverse events, substantiated LLLT as a safe and effective treatment for DLSO. Furthermore, the data demonstrate that LLLT was effective at treating varying degrees of DLSO. In more severe cases of DLSO, with an accumulation of keratinous debris, it might be expected that light attenuation would dampen the clinical effect. Nevertheless, the successful treatment of nails with an initial percent involvement over 90% and marked keratinous debris indicated the laser's ability to permeate the outer structure and successfully penetrate down to the nail bed, even without nail debridement. However, to understand the full utility of this procedure, further 12-month follow-up data are required to evaluate the rate of reoccurrence and sustainability of the treatment.

Mainstay therapies treat OM by targeting and downregulating the cytochrome P450 enzyme 14-α-sterol-demethylase or squalene epoxidase, two enzymes responsible for catalyzing sterol biosynthesis. Nevertheless, secondary considerations, such as impaired peripheral circulation, may reduce treatment success by limiting drug bioavailability. Additionally, aberrant peripheral circulation can impair the body's immune response and lead to hypoxia, creating an environment conducive to infection [25]. Further obstructing the body's immune response, dermatophytes, such as *Trichophyton rubrum, Trichophyton mentagrophytes,* and *T. mentagrophytes*, release exoantigens (or mannan) to evade innate immunity [26,27]. Moreover, conidia (non-motile fungal spores) ingested by macrophages differentiate and induce cell death after 8 h of growth [26]. Current mycosis treatments fail to address the complex pathogenesis of OM.

The two discrete laser light wavelengths used here have been reported to cause specific biological outcomes that are believed to provide a multifaceted treatment for DLSO. First, 635 nm (within the red visible spectrum) has been shown to activate PI3 kinase / eNOS signaling pathways, and to induce endothelial cell migration and neovascularization [28-30]. Furthermore, red light has been shown to improve phagocyte function and to induce a respiratory burst in neutrophils [21]. Conversely, 405 nm has been demonstrated to have an antimicrobial effect by upregulating the production of ROS, leading to the generation of hydrogen peroxide, hypochlorous acid, and hydroxyl radicals [31,32]. When applied concurrently, the combined antimicrobial and biostimulative effects appear to provide a therapeutically beneficial combination, as demonstrated by the mean percent changes in clarity.

A potential phototarget for the 405 nm wavelength is also a system responsible for catalyzing the generation of ROS, nicotinamide adenine dinucleotide phosphate oxidase (NOX) [31,32]. NOX transfers electrons from cytosolic NADPH to flavin adenine dinucleotide (FAD), then to extracellular molecular oxygen to generate superoxide [33,34]. The third and fifth transmembrane domains of NOX bind two prosthetic heme groups that shuttle electrons from FAD to oxygen [33,34]. It has been suggested that the prosthetic heme, which has been recognized as a photosensitizer, responds to the delivery of blue light. Stimulation of NOX could potentially provide two benefits: first, phagocytes are activated, and second, dermatophytes are susceptible to the toxic effects of ROS. Furthermore, squalene epoxidase, the therapeutic target for numerous antifungal medications, depends on the presence of NADPH or NADH and uses FAD to shuttle electrons from NADPH cytochrome P450 reductase. Loosely binding with FAD, SE may be subject to functional aberrations after light exposure [35].

Conclusions

Although several studies have demonstrated effects of both 635 nm and 405 nm laser light, the exact mechanism of action remains unclear. Nevertheless, the clinical data reported show the utility of dual-wavelength laser light in the treatment of varying intensities of DLSO.

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Table 1. Clear nail (mm) at each study evaluation point (n = 105).

mm clear nail (n = 105)	Baseline	2 months	3 months	6 months
Mean	4.6	7.62	9.59	10.75
Std. dev.	2.35	2.85	3.47	3.78

Table 2. Across each study evaluation point, analyses of the correlated samples showed a statistically significant clearance of the nail plate.

Evaluation Points	p-value
Baseline to 2 months	$p < 0.01$
Baseline to 3 months	$p < 0.01$
Baseline to 6 months	$p < 0.01$
2 months to 3 months	$p < 0.01$
2 months to 6 months	$p < 0.01$
3 months to 6 months	$p < 0.01$

Table 3. Mean change (mm) in correlated samples from baseline to 3 and 6 months for each baseline category (n = 105)

Category	3 mos. (mm)	p-value	6 mos. (mm)	p-value
< 25%	4.93	$p < 0.0001$	5.68	$p < 0.0001$
26-50%	5.31	$p < 0.0001$	6.51	$p < 0.0001$
51-75%	4.75	$p < 0.0001$	6.28	$p < 0.0001$

Table 4. Percent mean change of correlated samples from baseline to 3 months post-treatment for the entire population (*n* = 105)

Evaluation Point	Mean Change (%)	p-value
3 months	30.4	p < 0.0001
6 months	36.3	p < 0.0001

Table 5. Percent mean change from baseline to 3 and 6 months for each baseline category (*n* = 105)

Category	3 mos. (%)	p-value	6 mos. (%)	p-value
< 25%	39.4	p < 0.0001	46.6%	p < 0.0001
26-50%	36.4	p < 0.0001	42.1%	p < 0.0001
51-75%	18.3	p < 0.0001	23.6%	p < 0.0001

Table 6: Distribution of subjects according to observed mean percent nail clearance reported at month 6 (n =105)

Mean Change Reported	% of subjects
>25%	76.2
10-25%	18.1
<10%	5.7

Figure 1. Graphical representation of the percent clearance across each study evaluation point for the total population (n = 105).

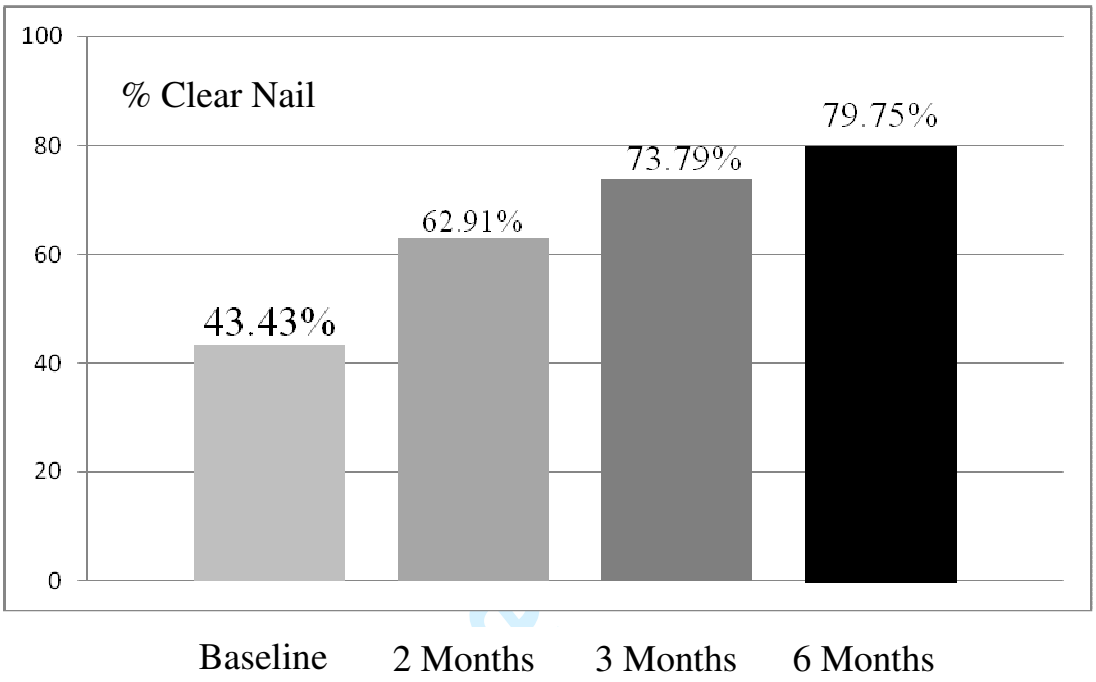
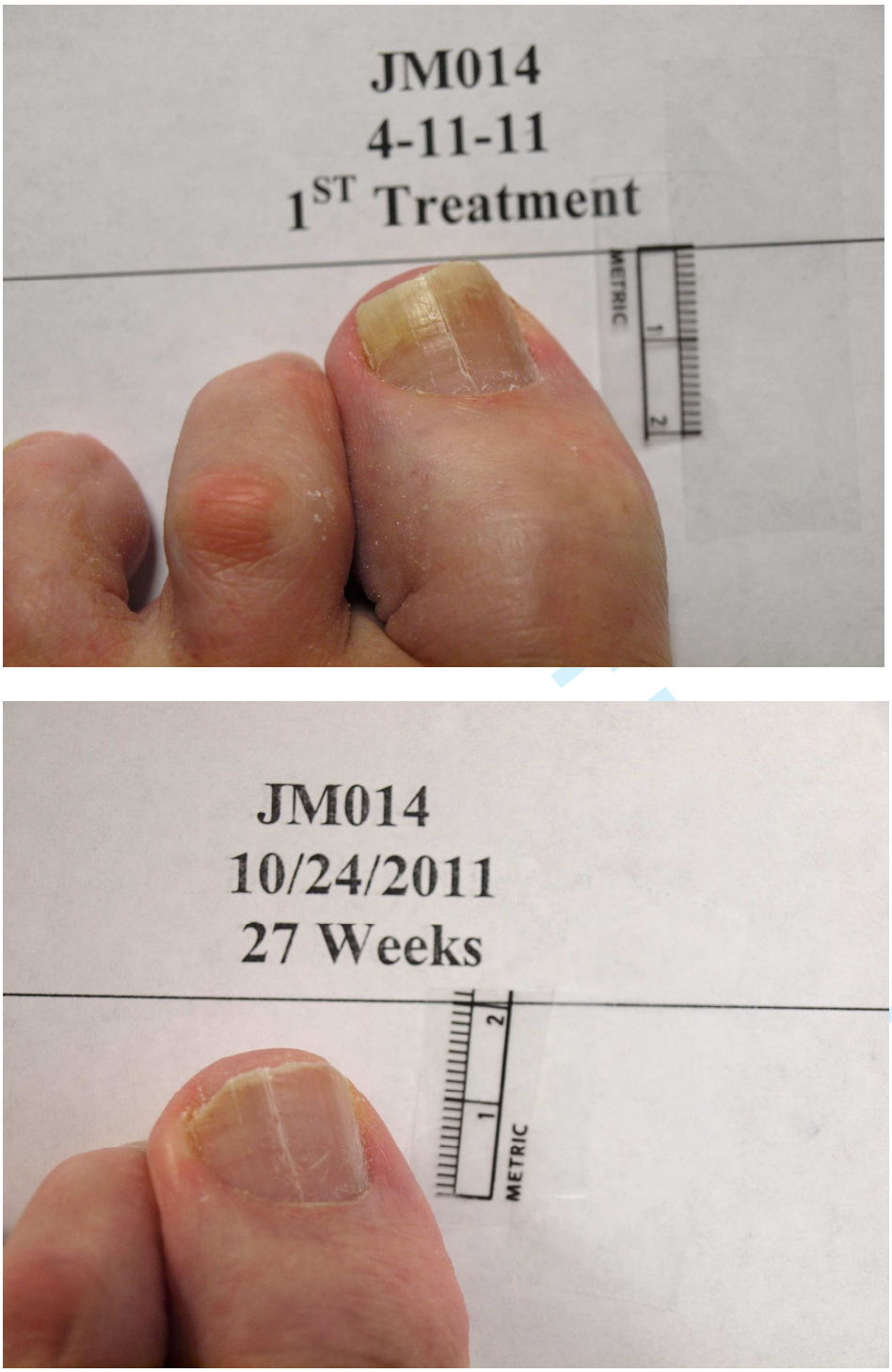
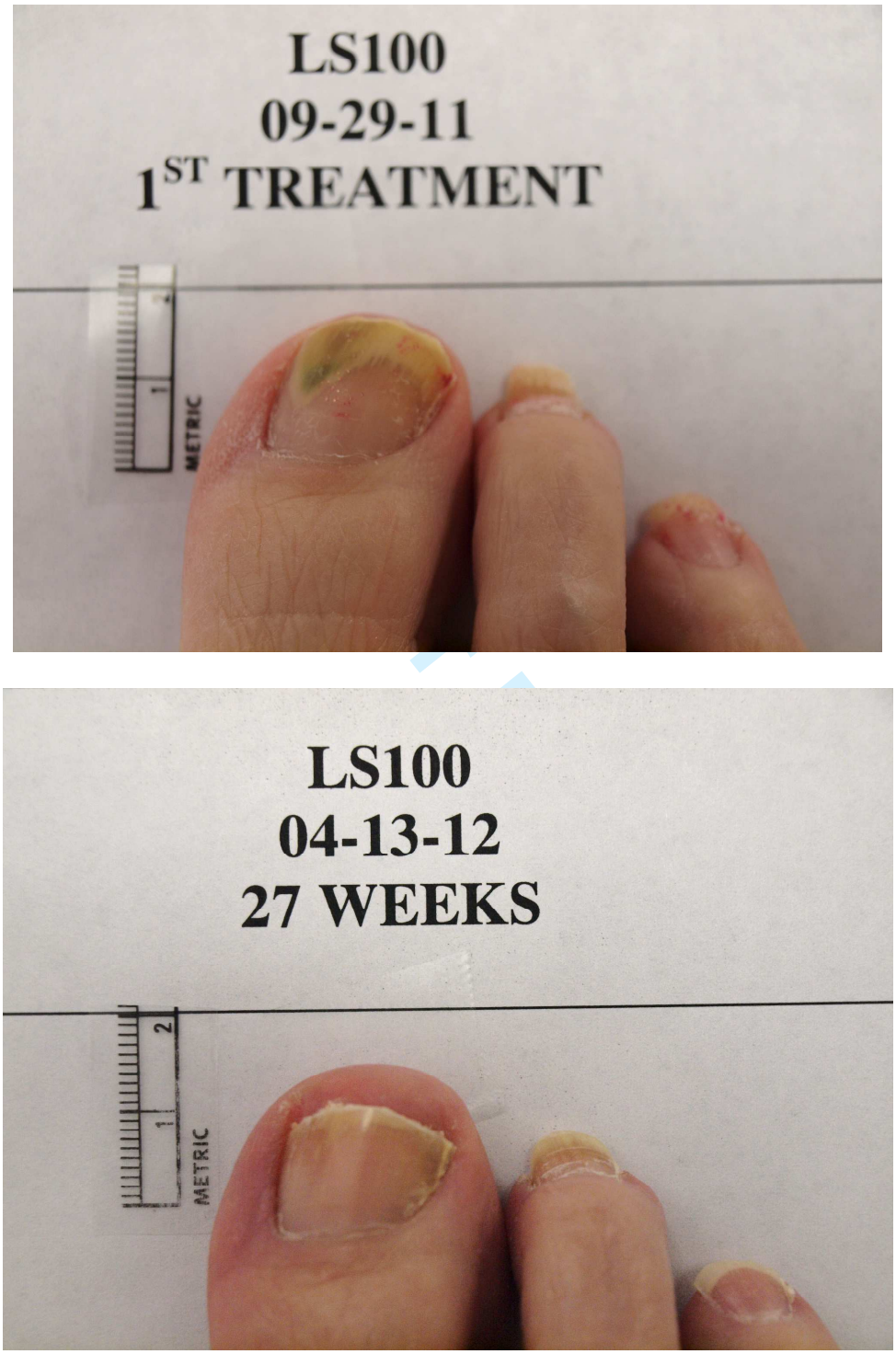


Figure 2. Before and after images for the 51-75% baseline category



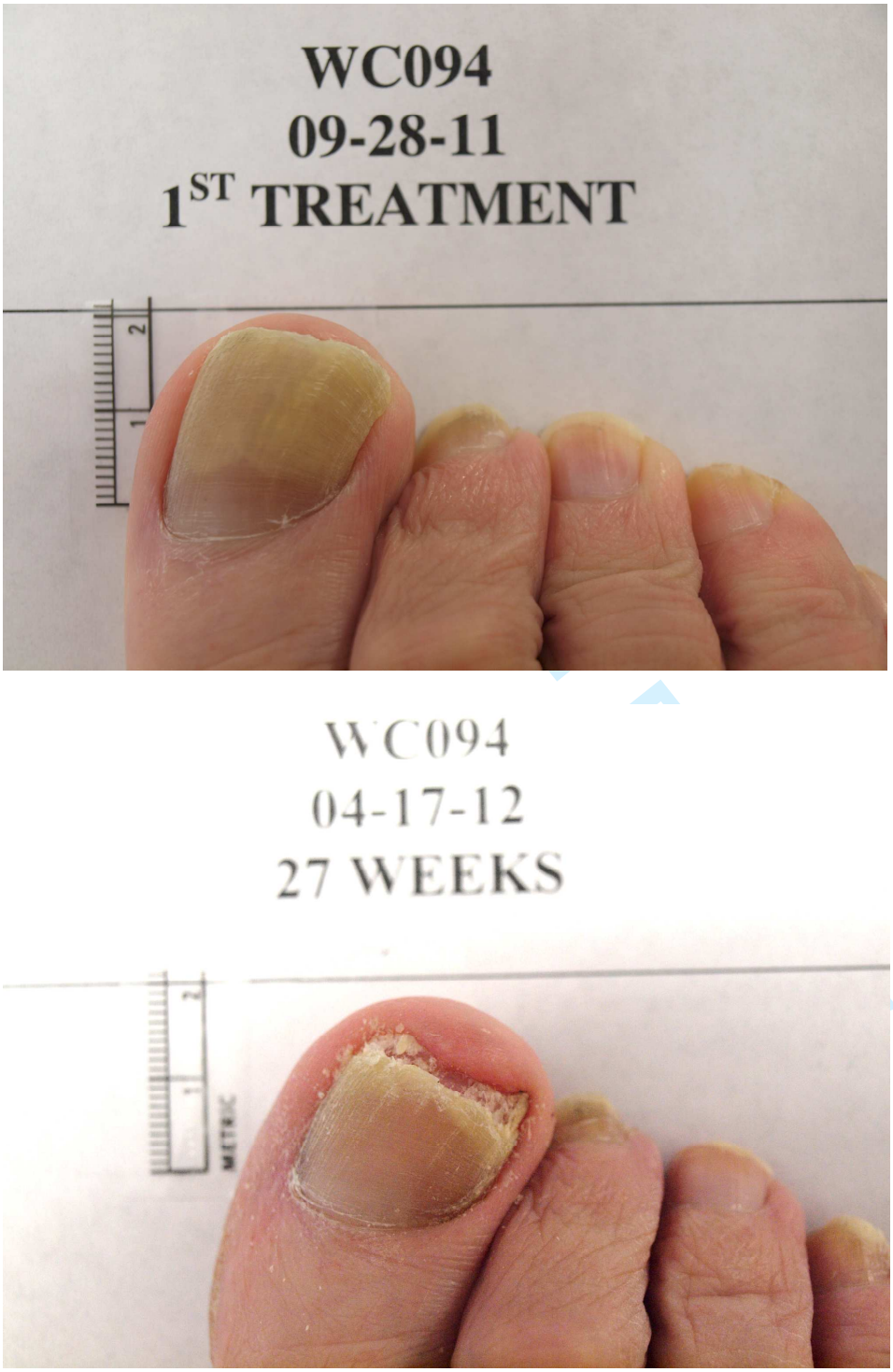
The percent mean change was 100%

Figure 3. Before and after images for the 26-50% baseline category



The percent mean change was 100%

Figure 4. Before and after images for the < 25% baseline category



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2 months to 6 months	$p < 0.01$
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< 25%	4.93	$p < 0.0001$	5.68	$p < 0.0001$
26-50%	5.31	$p < 0.0001$	6.51	$p < 0.0001$
51-75%	4.75	$p < 0.0001$	6.28	$p < 0.0001$

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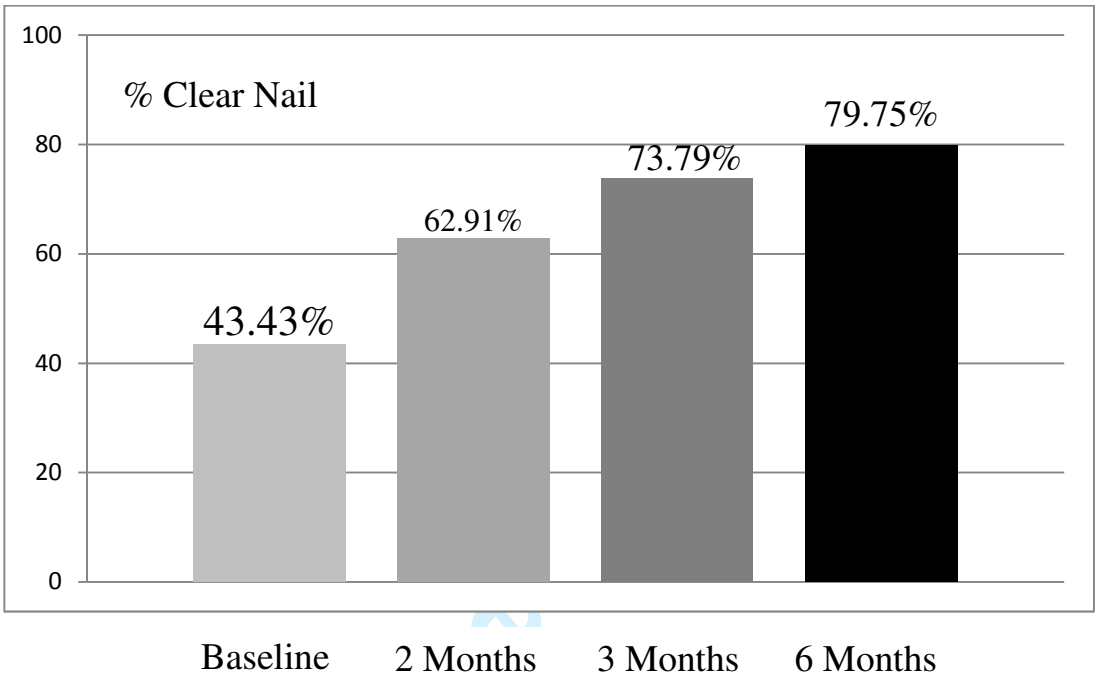
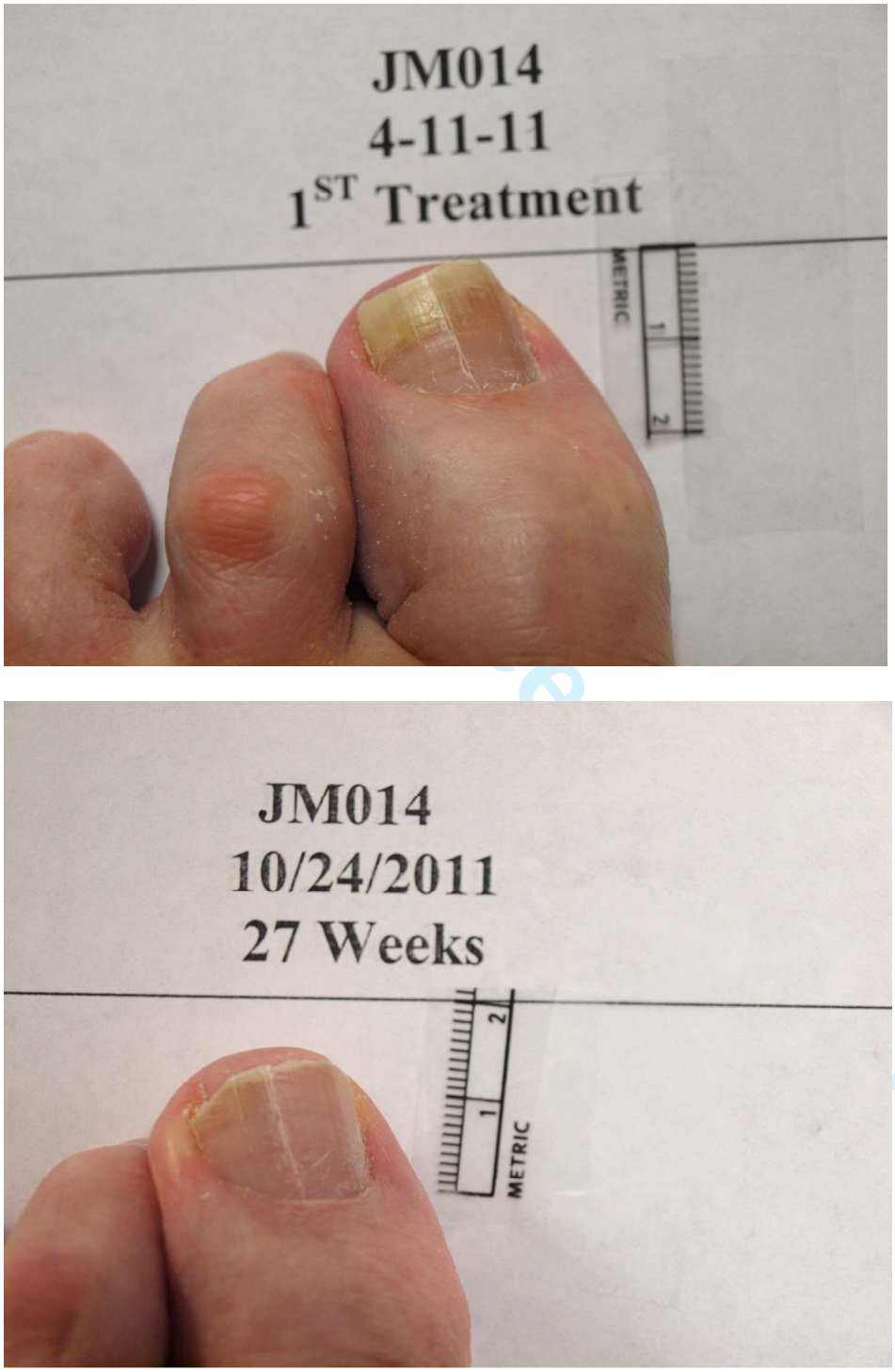
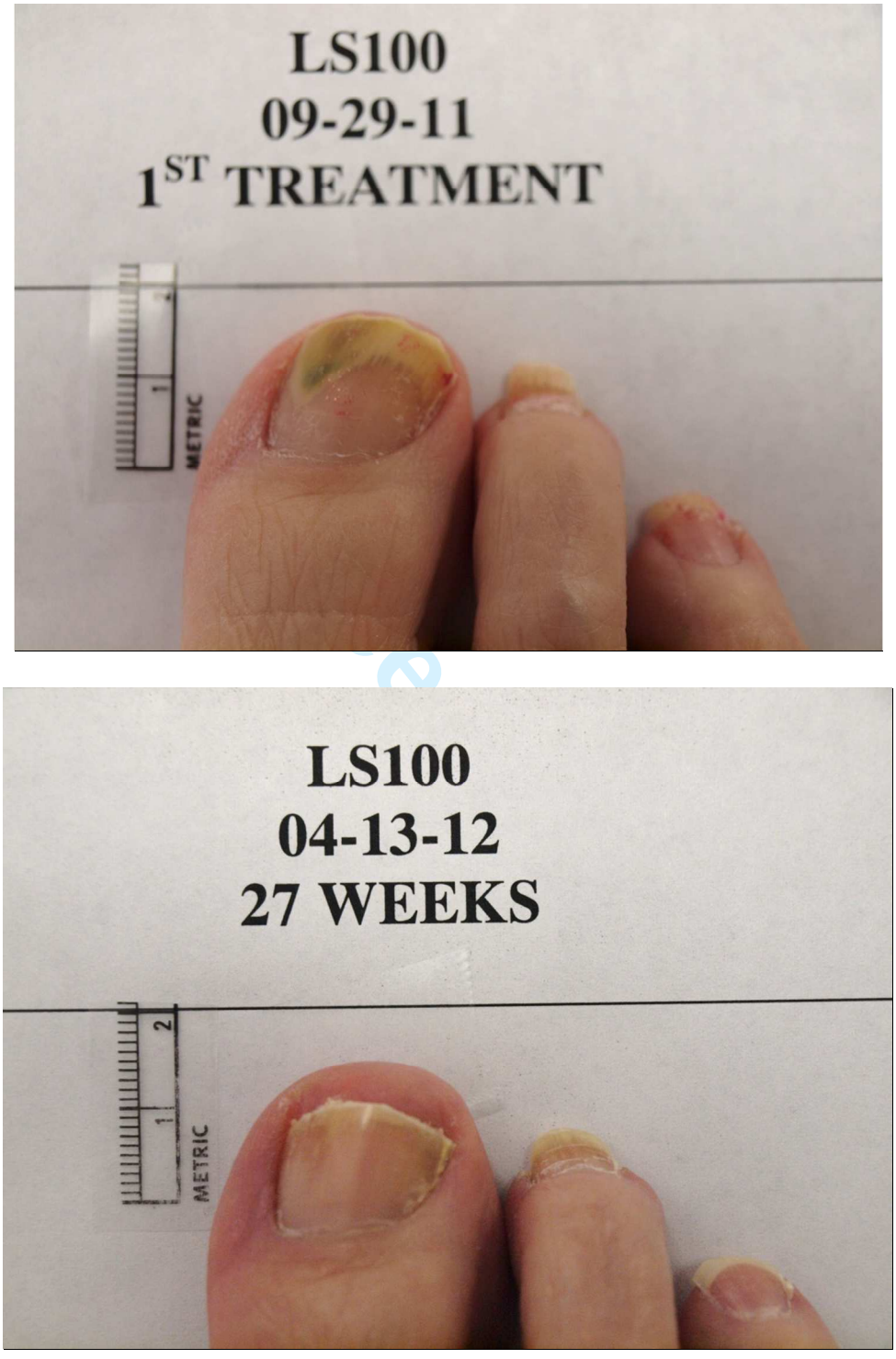


Figure 2. Before and after images for the 51-75% baseline category



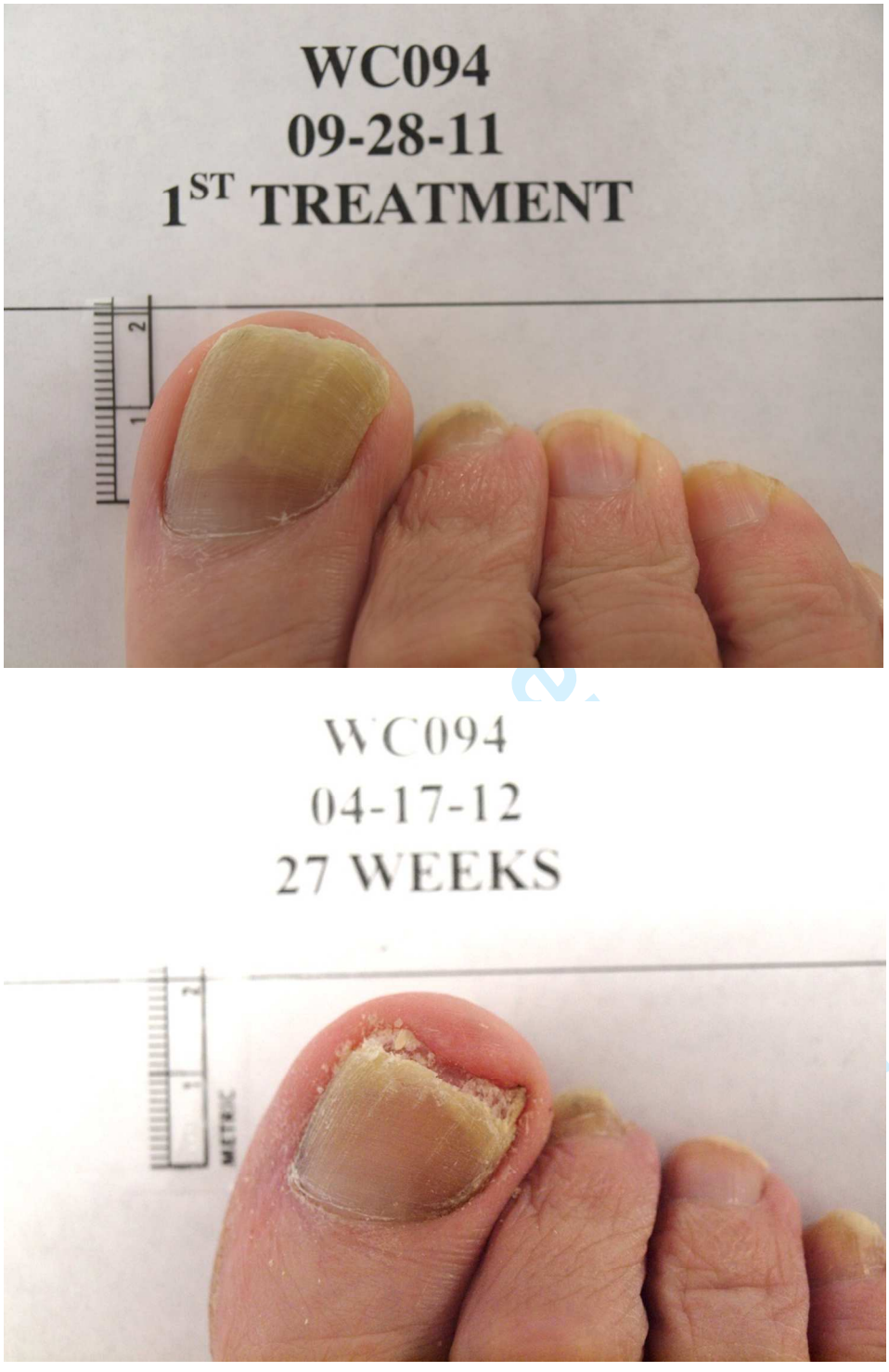
The percent mean change was 100%

Figure 3. Before and after images for the 26-50% baseline category



The percent mean change was 100%

Figure 4. Before and after images for the < 25% baseline category



The percent mean change was 100%