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Laser therapy for onychomycosis in patients with diabetes at risk for foot ulcers: A randomised, quadruple-blind, sham controlled trial (LASER-1)

Running title: Laser for onychomycosis in patients with diabetes

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Conflicts of interest

One of the employers of L. Nijenhuis-Rosien had a stake in a company that supplies medical devices, among others; the laser used for this study, L. Nijenhuis-Rosien has no stake in this company.

The other authors have no conflicts of interest to declare.

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Trial registration number

NCT01996995

Abstract

Backgrounds

Patients with diabetes mellitus are at high risk for onychomycosis, which is related to development of foot ulcers.

Objective

The aim of this study was to evaluate the safety and efficacy of the treatment of onychomycosis with local laser therapy.

Methods

In a single-centre, randomised (1:1), quadruple blind, sham-controlled trial, patients with diabetes mellitus, at risk for developing diabetic foot ulcers (Sims classification score 1,2)

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and a clinical suspicion on onychomycosis were randomised to either 4 sessions neodymium-doped yttrium aluminium garnet (Nd-YAG) 1064nM laser or sham treatment. The primary outcome was clinical and microbiological cure of onychomycosis after one-year follow-up.

Results

From March 2015 to July 2016 64 patients were randomised; 63 could be analysed. Trichophyton rubrum was the most detected pathogen. There was no difference in the primary outcome between laser and sham treatment. With the exception of a subungual hematoma in the fifth toenail occurring 2 weeks after laser treatment, the results suggested that treatment with Nd-YAG 1064nM laser is safe.

Conclusion

At this moment, there is no evidence of any effect of laser treatment for onychomycosis in patients with diabetes at increased risk for foot ulcers, at least not within one year after treatment.

Keywords

Onychomycosis, fungal toenail infection, laser, diabetes mellitus, diabetic foot

Abbreviations

Nd:Yag	Neodymium-doped yttrium aluminium garnet
DM	Diabetes Mellitus
PAD	Peripheral arterial disease
HRQOL	Health-related quality of life
FDA	Food and Drug Administration
ABI	Ankle Brachial index
AE	Adverse event
SAE	Serious adverse event

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OSI Onychomycosis severity index

PS Protective sensation

Introduction

Onychomycosis is a fungal infection of the nail plate characterized by discoloration, subungual hyperkeratosis thickening and sharpening of the nails and onycholysis [1]. In patients with diabetes mellitus (DM), the prevalence of onychomycosis and tinea pedis is 2.5 to 2.8 times higher[2-4]. Tinea pedis often lead to skin fissures and secondary infections. Onychomycosis can lead to skin injuries itself. Thickened, sharp brittle nails can pierce into the skin or lead to increased subungual pressure [4, 5], which are potential ports of entry for pathogens[6].

In patients with neuropathy and / or peripheral arterial disease (PAD), onychomycosis is an additional risk factor for foot ulcers, cellulitis, osteomyelitis and gangrene[7-10]. Diabetic foot ulcers are a major cause of hospitalizations and amputations, and loss of health-related quality of life (HRQOL) [11-15].

Treating onychomycosis is recognized as a potential strategy for preventing DM related foot complications [4, 16-18]. Usual care for onychomycosis is symptomatic: every six weeks nails are skived to normal proportions and sharp edges are removed. Effective systemic antifungal agents are available, but are often withheld to patients with DM due to concerns of side effects and medication interactions [19].

Local laser therapy is also used for the treatment of onychomycosis [20]. Laser therapy is a Food and Drug Administration (FDA) approved therapy for improving toenail appearance [20]. Laser systems are presumed to have predictable adverse effects[20, 21]. In vitro studies of laser effects showed a fungicide effect on *Trichophyton rubrum* after 10 minutes at 50°C [22]; pathogen growth was inhibited only temporarily using high fluences [23]. This notwithstanding,

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there are few non-randomised or randomised studies investigating the effects of laser therapy on onychomycosis.

To our knowledge, none had a triple or quadruple blind design, and none were performed in patients with DM who also had additional risk factors for foot ulcers. Primary aim of this trial was to evaluate safety and complete cure rate of onychomycosis of laser therapy in patients with diabetes and an increased risk for foot ulcers.

Materials and Methods

Trial design

This study was a single-centre, randomised (1:1), quadruple blinded, sham-controlled trial in patients with diabetes mellitus and a higher risk on foot ulcers [24]. This manuscript was written in accordance with the CONSORT statement[25].

Participants

Patients were eligible to participate when ≥18 years, had type 1 or 2 diabetes, had risk factors for developing foot ulcers (defined as Sims classification 1 or 2), and a clinical suspicion of onychomycosis. The study was performed at the outpatient podiatric foot clinic from Innofeet in Isala (Zwolle, the Netherlands).

Exclusion criteria were having no microbiologic confirmation, Sims classification 0 or 3, ischaemic rest pain in a leg, ankle-brachial index <0.9, a toe pressure < 50 mmHg, having renal replacement therapy or severe renal insufficiency (estimated glomerular filtration rate (eGFR) <30 ml/min/1.73m^2), use of systemic or topical antifungal agents 3 months prior to inclusion, use of immunosuppressive drugs, presence of psoriasis, lichen planus, or other abnormalities potentially involving the toenails, a history of epilepsy, and insufficient knowledge of the Dutch language. Patients with skin colour Fitzpatrick 4 and 5 were

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excluded since darker nails theoretically may lead to increased temperatures during laser application [26].

In the Sims' classification, Sims 0 is defined as no loss of protective sensation (PS) or no presence of PAD; Sims 1: loss of PS and / or PAD, without signs of locally increased pressure; Sims 2: loss of PS and/ or PAD in combination with signs of locally increased pressure (e.g. local redness, callus, bony prominence). Sims 3: a previous ulcer, Charcot foot or amputation. Presence of peripheral neuropathy was tested using a 10g (5.07 Semmes-Weinstein) monofilament. Presence of peripheral arterial disease was examined by measuring the ankle-brachial index (ABI) using a handheld Doppler (Huntleigh D9000, EZ8 probe). Systolic blood pressure in the arm was measured using a handheld Doppler at the A. brachialis, systolic blood pressure in the leg was measured at the A. tibialis posterior.

Interventions

During the study, patients visited the clinic 7 times. The primary investigator (L.N.) performed baseline measurements, nail sample collection and determined eligibility for inclusion. From every patient who gave written informed consent and who met the inclusion criteria, a nail sample was taken.

The largest affected toenail was identified as target nail. The target nail is the nail with the highest Onychomycose Severity Index (OSI) [27] and the largest surface. All nails were disinfected with alcohol 70% previously to sampling, to remove contaminants. Specimens were kept in dry media to avoid rapid multiplication of bacterial and fungal spores[28]. Because the nail sample has to be dry scraping dust of the affected nail, the milling machine from the podiatrist was used without water-cooling. For each single patient a sterilized micro drill was used. In the proximal part of the nail at the transition of abnormal and normal part of the nail, a sample was taken, since this is the most appropriate place for finding the most viable

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hyphae in this part of the nail. Within an hour after obtaining the specimen, it was transported to the microbiological laboratory of Isala.

During 2th- 5th visit patients were seen by podiatrists (M.A.E.M and A.B.S) who performed the randomisation and applied the treatment.

The microbiological laboratory performed its evaluation using standard methods[29]. Each specimen was evaluated by blankophor examination, culture and PCR. The nail dust was seeded for culture on two different Agars; Sabouraud maltose agar (Oxoid CM0541) + amoxicillin/gentamicin (SAB MAL + AG) and Malt extract agar + saccharose / chloramphenicol cycloheximid (MOS-1). Direct microscopy was done with the addition of the optical brightener Blankophor-P. Blankophor-P was added at a potassium hydroxide (KOH) solution (25 mg Blankophor-P + 100 ml KOH 30%). Blankophor-P is binding at the chitin in the cell walls of the fungus and yeasts. With a 425Nm filter fluorescence microscope all specimens were assessed for the presence of pseudohyphae, hyphea and/ or yeasts. Only patients with positive blankophor, culture, or PCR were included in the study and randomised. All patients in both groups were instructed with regard to hygiene measures to prevent re-infections.

Before starting with (laser or sham) treatment, all affected nails were grinded with a high-speed nail grinder (Bentlon, Podospray Gold S2 Podiatry drill) by the podiatrist.

All nails were treated with either laser or sham procedure at baseline, and after 2,4 and 12 weeks. In the laser group two passes were applied continuously over the entire area of the nail (nail plate, nail fold and eponychium) in a grid pattern. The spot (laser dot) overlapped the edge of the previous row of pulses.

The technical treatment parameters of the laser were: wavelength 1064 nM, fluency 20 J/cm^2; spot size 3 mm; pulse rate of 5 Hz; power of 10 W and pulse duration of 132 ms. All Serious Adverse Events (SAE) were reported to the Medical Ethical Committee (METC).

At baseline, t=12, t=30 and t=52 weeks pictures were taken from all nails. Each target nail was photographed, using a Panasonic Lumix DMF-FX40 12 megapixels camera, perpendicular to the nail (see figure 1) and the distal nail top (see figure 2) on a surface with a

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1*1cm grid with a ruler with 1mm subdivision scale next to the nail. Three independent assessors (1 podiatrist, 1 general doctor and 1 internist) judged all pictures to determine the OSI at baseline and week 52. The two pictures were presented separately (with three months in between) so no comparison between the two pictures could be made. The average of the three assessments was used for analyses. The percentage of nail involvement was assessed using the digital images of the nails and the software Image J, since the inter-and intra-reliability of Image J is very high[30].

[figure 1] [figure 2]

Outcomes

Primary endpoint was complete cure rate of the target nail, being defined as a completely normal looking nail, or negative microbiological results in case only minor abnormalities were still present. A minor abnormality was defined as an irregularity of the nails involving <5% of the surface area of the target nail at less than ¼ of the distance of the distal nail edge, and without hyperkeratosis at week 52. *Secondary endpoints* included the microbiologic cure rate of the target nail, complete clinical cure of the target nail, onychomycosis severity index below 6 (in patients with scores >6 at study entry) of the target nail, change in the affected surface area, absence of subungual hyperkeratosis and changes in HRQOL between groups over time.

Patients were regarded as having a persistent infection when baseline, week 30 and week 52 microbiological results showed the same organism. Patients with a same species infection at baseline and in week 52 but negative biological results at week 30 were regarded as having a recurrent infection. Patients in whom different organisms were isolated at week 30 or 52 compared to baseline were regarded as having a new infection.

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A ‘markedly improved target nail’ was defined as a nail with less than 10% abnormalities and without hyperkeratosis after 52 weeks, when initially more than 10% was affected.

Sample size

Sample size was calculated by using the computer program G-power 3.0. Estimating the proportion of patients with a complete cure to be 40% and 5% in the control, with a power of 0.85, alpha 0.05, 2-tailed, the total sample size would be 56. For possible loss to follow-up the total sample size was increased to 64.

Randomisation

Randomisation was done in blocks (5 blocks of 10 and 1 block of 14) by a third party using sealed, non-transparent envelopes. The podiatrists who performed the treatment received a sealed envelope with an x or y on the paper. Patients were not told which treatment they received.

Implementation

L.N. enrolled participants, performed baseline and week 52 measurements..

A.B.S and M.A.E. M performed all (sham) treatments of the patients and 30-week controls.

N.K supervised the randomisation.

S.H.A. D, J.H and D.S assessed all pictures for the OSI.

K.H.G performed all statistical analyses.

Blinding

The investigators, patients, outcome assessors and statistician were blinded for allocation. During the study two separate podiatrists who were otherwise not involved in the study

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performed the laser and sham treatments (A.B.S and M.A.E.M). Before starting the treatment of the laser or placebo the patients were blinded by inserting a hanging barrier (a plain cloth) between the patients' head and his or her feet. In addition, a blinded laser safety goggle was worn by the subjects. During the procedure the laser was actually turned on but blasted in a fireproof dish, which was placed next to the foot. The procedural sounds and lights during the laser application and sham procedure were identical.

Statistical methods

Data entry was performed in duplicate. All statistical calculations were performed using SPSS 23 (IBM). A 5% significance level was used. Analyses were performed according to the intention-to-treat-principle. The Fisher's exact test was applied for categorical variables and to test for differences between the treatment groups. In clinical and microbial cure rates 95% confidence intervals (CI's) were constructed. The non-normally distributed continuous variables were compared with the Mann-Whitney-U test.

Ethical considerations

Each patient gave written informed consent before participating in this study. This trial was approved by the Medical ethics committee Isala, Zwolle *NL46084.075.13/METC no. 13.0885.

Results

Participant flow

As shown in Figure 3, one patient in the sham group was excluded after randomisation because baseline blankophor, culture and PCR proved to be negative.

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Recruitment

From March 12 2015 to July 7th 2016 113 patients were invited to participate. Sixty-four patients were randomised. The last patient was evaluated July 6th 2017.

Baseline data

As presented in Table 1 the 63 patients (34% female) included in this study had a mean age of 68 (SD 10) years, DM duration of 16 (SD 9) years; 50 patients (80%) had type 2 diabetes. In 34 patients (54%) the target nail was located on the left foot. Most target nails were on the first toe; 28 (90%) in the sham group versus 30 (93%) in the intervention group. Prior to the study, (but >3 months before entering the study), 43 patients (68%) used any anti-mycotic agent. At baseline, mean OSI was 25.7 (SD 6.6).

In both groups *Trichophyton rubrum* (intervention 78%, sham 71%) was the most detected pathogen (Table 2).

Numbers analysed

From 64 participants, 63 were analysed (Laser group n=32, sham group n=31)

No patients were lost to follow-up.

Outcomes

No participant reported knowing about his or her treatment group.

Two patients reached primary outcome. Both of these patients were in the treatment group. The difference between the laser and sham group was not significant.

27(42.9%) Patients reached a microbiological cure, 13(41.9%) in the sham group (95%CI: 26.4%, 59.2%) and 14(43.8%) in the intervention group (95%CI: 28.2%, 60.7%, p=1.00. No patients reached a complete clinical cure of the target nail. Ten patients showed a markedly

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improved nail: three patients in the sham group (95%CI: 3.4%, 24.9%) and seven in the intervention group (95%CI: 11.0%, 38.8%). Again the difference was not significant.
Of the patients with a OSI > 6 at entry of the study (n=62) 14 had a decrease, four remained stable and 44 had an increase in the OSI during the study. One patient in the intervention group had a decrease of the OSI to < 6.
Surface involvement of the target nail in the sham group was 66.2% (SD 22.4) at baseline and decreased to 56.5% (SD 23.72) at week 52 (difference: -9.7, 95%CI: -16.8, -2.5). In the intervention group there was a decrease from 73.6% (SD 20.5) to 60.6% (SD 28.6) (difference: -13.0, 95%CI: -20.8, -7.2). The difference in decrease between the intervention and sham group was 3.3% (95%CI: -6.4, 13.0). In the sham group the mean OSI decreased from 22.9% (SD 7.0) to 19.6% (SD 7.1) (difference: -3.3, 95%CI: -5.4, -1.2), in the intervention group from 24.7% (SD 6.1) to 20.2% (SD 10.0) (difference: -4.5, 95%CI: -7.5, -1.5). The difference in decrease between intervention and sham group was 1.2% (95%CI: -2.4, 4.82). None of these differences were significant. In the sham group one patient vs none in the intervention group had a new infection (p=0.492).
Recurrence was seen in three patients of the sham group and in six of the intervention group (p=0.474). A persistent infection occurred in 14 patients in the sham group and 13 in the intervention group (p=0.801). In both groups 13 patients had a therapy success (p=1.0)(Table 3).

Safety / harms

During the study there were seven serious adverse events (SAE) and 2 adverse events (AE). Six SAE's were in the intervention group. All these events were unrelated hospital admissions. Both adverse events were seen in the intervention group. One patient fractured her first and second toe (contralateral of target nail side). The other patient reported a

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subungual hematoma on digit five, two weeks after treatment session 2. Due to neuropathy he could not exclude a trauma. Four (12.9%) patients in the sham group reported pain during the treatment versus three (9.4%) patients in the laser group. Six (19.4%) patients in the sham group and ten (31.3%) in the laser group considered the treatment tender

Discussion

In this randomised quadruple blind, sham-controlled trial, there were no significant differences in complete and microbiological cure rate of onychomycosis between 4 sessions of laser treatment and a sham procedure in patients with diabetes at high risk for developing diabetes related foot complications. There was one subungual hematoma; a relationship with the laser procedure could not be excluded. Due to his neuropathy a trauma could not be excluded. The results suggest that 4 treatment sessions with Nd-YAG 1064nM laser is not effective; no serious safety issues came up.

Previous studies on the treatment of onychomycosis with 1064 Nm n:YAG laser showed contradictory results. Studies are difficult to compare because of differences in study design, study population and treatment protocols. One prospective non-randomised study with 35 patients showed an overall cure rate of 51.9% in patients after 6 months follow-up. 70% of the studied population had a skin type Fitzpatrick IV; the most frequently isolated pathogens were *Scytalidium dimidiatum* and *Trichophyton mentagrophytes* [31]. A study from Kimura et al in 13 patients with 1-3 treatments in 4-8 weeks and a 16-week follow-up showed partial or complete curation rate of 81%[32]. Earlier randomised clinical studies didn't show treatment effects. In a non-diabetes population, Hollmig *et al.* found no significant differences in mycological culture or clinical nail plate clearance with 2 sessions (with a 2 week interval) of Nd-YAG 1064nM laser treatment compared to controls [33]. Karsai *et al.* compared four laser treatments (at 4-6 week intervals) to no laser treatment in 20 non-diabetes patients (82

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mycotic nails); there were no mycological remissions after 12 months [34]. Furthermore, they did not find differences in change of OSI or pain scores.

The results of the present study and other studies in non-diabetes populations suggest that laser therapy is not effective in treating onychomycosis. More intensive therapy could influence effectiveness, but no studies are known. Due to the presence of protective sensation loss, part of the patients do not register high temperatures which might result in burning [35]. Furthermore, it could be that one-year follow-up is not enough to properly assess the extent of the cure. Some patients in the sham group showed clinical improvement of the nails. All patients received usual care. Before treatment, the nails were skived 4 times (before every treatment/ sham procedure) and patients were able to treat fungal skin infections themselves by applying antifungal cream (like miconazolnitrate), which could have cured superficial onychomycosis.

Strength of this study is the quadruple blind design, the sham-controlled group and the blinding procedure. Most patients were not employed and the average age of the study participants was over 65 years. Since a higher age is associated with lower cure rates, age could have influenced the outcomes of this study [36]. A normal toenail grows out completely after 12-18 months[37], but in the total study group the growth rate of the nails appeared to be lower. Futures studies might gain by extending the follow–up period. Nevertheless, the risk of recurrence and new infections will probably be also higher during a longer follow–up period. Therefore, the eventual effect of study duration extension will be difficult to predict. Another limitation of our study is the lack of the ability to measure patient compliance with powdering socks and shoes. Finally, most of the patients in our study population had a onychomycosis with matrix involvement, subungual hyperkeratosis, hallux involvement,

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>50% nail involvement, all of which are prognostic factors associated with a lower overall success rate [36].

Conclusion

With one year follow-up, laser treatment for onychomycosis in patients with diabetes at risk for foot ulcers does not seem to be effective and should be regarded as experimental therapy. Whether other schemes of treatment intensity, treatment duration, of follow-up duration will yield other results could be focus of future research.

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Statement of assistance

GL and NK conceived the study. GL, LN and NK developed the study protocol. HB, PvD, MW, KG made critical revisions to the manuscript. MW supervises the microbiological study. KG performed the statistical planning. All authors have reviewed and approved the final version of the manuscript.

Conflicts of interest

One of the employers of L. Nijenhuis-Rosien had a stake in a company that supplies medical devices, among others; the laser used for this study, L. Nijenhuis-Rosien has no stake in this company.

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The other authors have no conflicts of interest to declare.

Registration

This study is registered on clinical trials.gov; NCT01996995

https://clinicaltrials.gov/ct2/show/NCT01996995?cond=laser+onychomycosis+diabetes&rank=2

Protocol

Our study protocol in free downloadable at https://doi.org/10.1186/s13063-015-0622-4)

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Table 1

Characteristic	Total N=63	Intervention N=32	Sham N= 31
Age at baseline (year)	67.8 [60.9,73.1]	70.5 [60.7,73.8]	66.3 [60.9,71.1]
Gender (Female)	21 (33.3)	12 (37.5)	9 (29)
BMI (kg/m^2)	29.9 [27.7,33.8]	29.1 [27.2,35.8]	30.0 [26.9,33.7]
Smoking yes (ever/current)	48 (75)/ 10 (15.9)	23 (71.9) / 3 (9.4)	25 (80.6) / 7 (22.6)
Smoking (PY)	7.5 [0.1,22.5]	2.5[0.0,20.0]	9.0 [0.3,23.6]
Alcohol use (yes)*	29 (46.0)	14 (43.8)	15 (48.4)
Diabetes (type 1/type 2)	13 (20.6)/ 50 (79.4)	7(21.9) / 25 (78.1)	6(19.3)/ 25 (80.6)
Diabetes duration (years)	15.7 [9.5,22.0]	14.5 [8.5,23.0]	15.7 [11.3,21.0]
Presence of micro vascular complication			
Neuropathy	63 (100)	32 (100)	31 (100)
Retinopathy	8 (12.7)	6 (18.8)	2 (6.5)
Nephropathy	5 (7.9)	4 (12.5)	1 (3.2)
Presence of macro vascular complication			
Angina pectoris	5 (7.9)	5 (15.6)	1 (3.2)
Myocardial infarction	6 (9.5)	5 (15.6)	1 (3.2)
PTCA	8 (12.7)	5 (15.6)	3 (9.7)
CABG	4 (6.3)	2 (6.3)	2 (6.3)
TIA	5 (7.9)	3 (9.4)	2 (6.5)

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CVA	5 (7.9)	4 (12.5)	1 (3.2)
Hba1c at baseline %	7.5 [6.7,8.2]	7.5 [6.5,8.2]	7.5 [6.8,8.2]
Hba1c at baseline in mmol/mol	58.0 [50.0,66.0]	58.0 [47.3,66.5]	58.0 [51.0,66.0]
Use of diet alone	3 (4.8)	2 (6.3)	1 (3.2)
Use of oral drugs alone	18 (28.6)	8 (25.0)	10 (32.3)
Use of Insulin alone	19 (30.2)	10 (31.3)	9 (29.1)
Use of any blood glucose lowering drugs (yes)	60 (95.2)	31 (93.9)	30 (96.8)
ABI left	1.3 [1.2,1.4]	1.3 [1.2,1.4]	1.3 [1.1,1.4]
ABI right	1.3 [1.2,1.4]	1.3 [1.2,1.4]	1.3 [1.2,1.4]**
Location of target nail (left/right foot)	34 (54.0) / 29 (46.0)	21 (63.6)/12 (36.4)	14 (45.2) / 17 (54.8)
Affected toenails	5.0 [2.0,7.0]	5.0 [2.0,7.0]	5.0 [2.0,7.0]
Use of any anti-mycotic before (yes)	43 (68.3)	22 (68.8)	21 (67.7)
Oral	7 (11.0)	4 (12.5)	3 (9.7)
Topical	42 (66.4)	21 (65.6)	21 (67.7)
OSI at baseline	25.7 [18.7,28.3]	26.7 [120.8,28.3]	23.7 [18.0,28.3]

Table 1: Table 1 Baseline Characteristics

Data is presented as number (%), mean (SD) or median [IQR]. Abbreviations: ABI: ankle-brachial index, BMI; body mass index, CABG; coronary artery bypass grafting, CVA: cerebrovascular accident, OSI: onychomycosis severity index, PTCA: Percutaneous trans luminal coronary angioplasty, PY; pack years, TIA: transient ischemic attack.

*missing n=2, ** missing n=1 could not be measured by mamma carcinoma in medical history.

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Table 2

	Total group Culture N (%)	**Total group PCR N (%)**	**Intervention Culture N (%)**	**Intervention PCR N (%)**	**Sham Culture N (%)**	**Sham PCR N (%)**
Trichophyton rubrum	20 (31.7)	47 (74.6)	10 (31.3)	25 (78.1)	10 (32.3)	22 (71)
Trichophyton mentagrophytes	4 (6.3)	13 (20.6)	2 (6.3)	6 (18.8)	2 (6.5)	7 (22.6)
Epidermophyton floccosum	-	1 (1.6)	-	1 (3.1)	-	-
Trichophyton interdigitale	1 (1.6)	-	-	-	1 (3.2)	-
Scopulariopsis brevicaulis	1 (1.6)	-	-	-	1 (3.2)	-
Trichosporon mucoides	1 (1.6)	-	1 (3.1)	-	-	-
Saccharomyces cerevisiae	1 (1.6)	-	1 (3.1)	-	-	-

Table 2; Detected pathogens at baseline

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Table 3

	Intervention group (n)	Sham group (n)	p-value Fisher's exact test
Persistent infection	13 (32)	14 (31)	0.801
Recurrence	6 (32)	3 (31)	0.474
New infection	0 (32)	1 (31)	0,492
Treatment success	13 (32)	13 (31)	1.000

Table 3; Outcomes after 4 sessions Nd-Yag laser

Figure 1

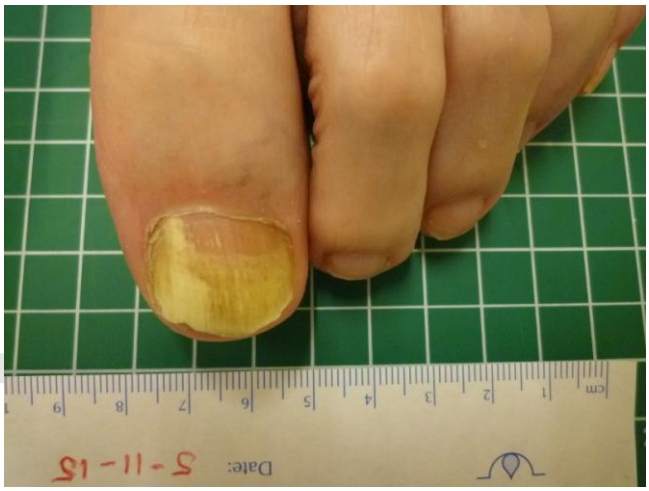

Figure 1: perpendicular to the nail. Permission to use these pictures has been obtained.

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Figure 2

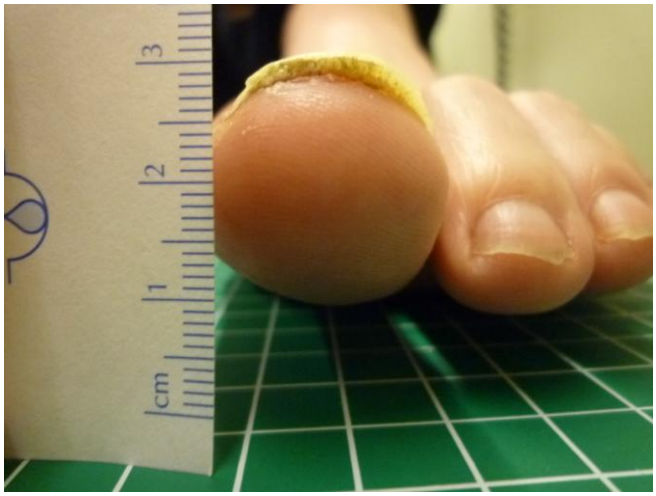

Figure 2: perpendicular to the nail top. Permission to use these pictures has been obtained.

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Figure 3 CONSORT FLOW CHART

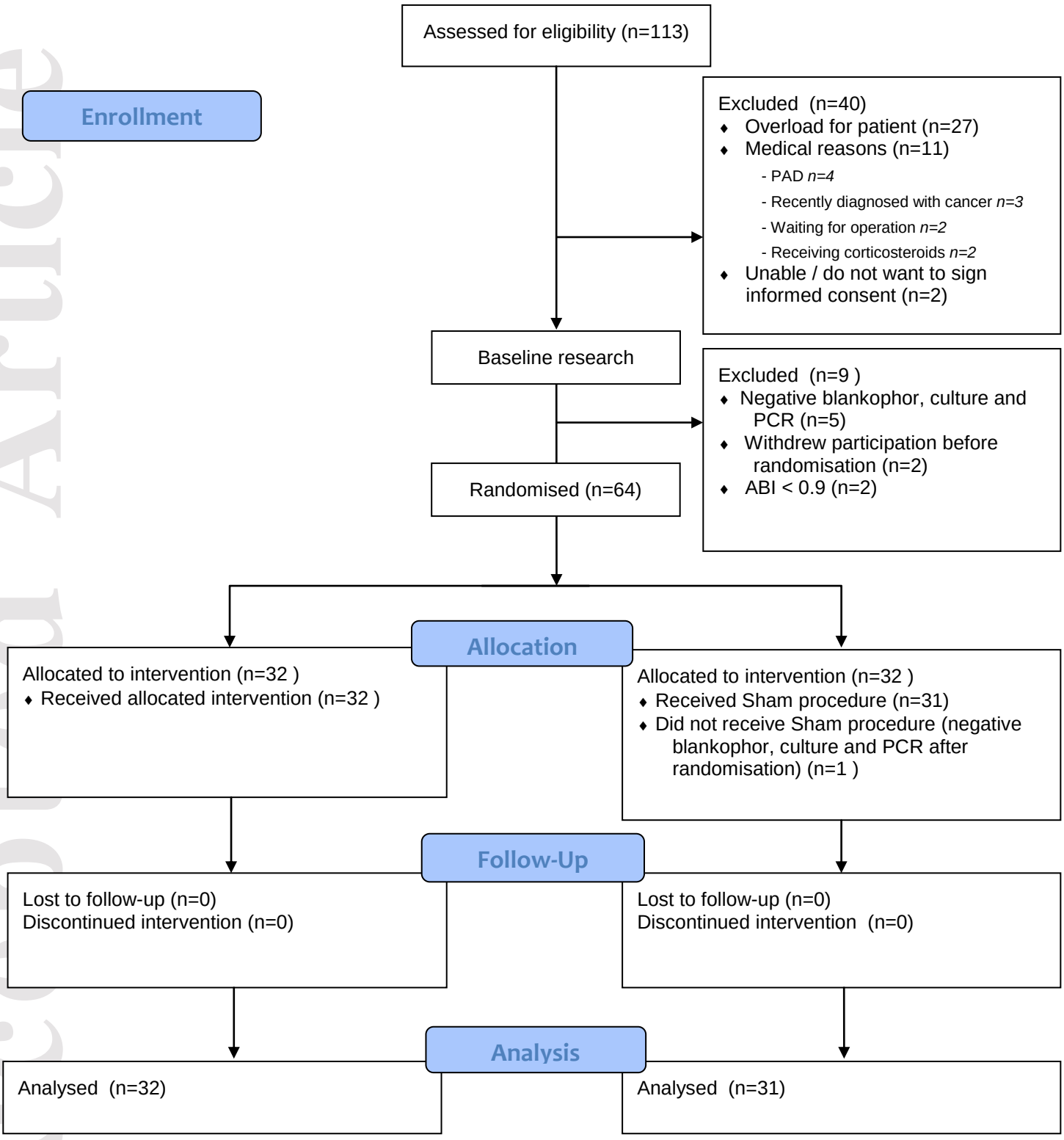

Figure 3: Consort flow chart

Figure 3: Consort flow chart. Abbreviations : PAD= peripheral arterial disease, PCR= polymerase chain reaction, ABI= Ankle Brachial Index

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