Nail acidification vs. amorolfine in the local management of onychomycosis: a comparative, prospective, randomized, blinded trial

F. Eertmans[1], N. Doss[2], B. Rossel[1], P. A. Regidor[3]

[1]Oystershell Laboratories, Drongen, Belgium
[2]Department of Dermatology, Université Tunis El Manar, Military Hospital of Tunis, Tunis, Tunisia
[3]Outdoor Department Frauenklinik München West, Frauenklinik, München West, Germany

ABSTRACT *— INTRODUCTION: Onychomycosis is a fungal infection, frequently caused by dermatophytes, that affects hand and foot nails. Infection rates in Western adult populations range from 2% to 14%, although up to 50% of people over 70 years of age may be affected. Prevalence of onychomycosis is also higher in immuno-compromised and patients with diseases that affect peripheral circulation, such as diabetes mellitus. The aim of the present study was to evaluate clinical efficacy of a nail acidifying solution vs. nail lacquer containing 5% amorolfine for the local treatment of mild to moderate nail onychomycosis.*

PATIENTS AND METHODS: 112 adults with confirmed onychomycosis (at least one great toenail) were randomized in this open, prospective, blinded trial. The acetic acid/ethyl lactate-based solution was brushed on twice-daily and the amorolfine lacquer applied and removed weekly for 168 days. Out of these 112 patients, a fully data analysis could be performed in 102 patients (53 acetic acid group and 49 amorolfine group, respectively). Clinical efficacy was evaluated at the following time points: day (D) D0 = baseline, D14, D28, D56, D112, and D168, respectively. All patients underwent microbiological testing at baseline and at the end of the treatment. Primary objective of this trial was the change in the percentage of healthy nail surface at study end.

RESULTS: The percentage of healthy surface between baseline and D168 increased with 11.4% (± 17.0%) in the acid-based treated patient group and 5.2% (± 12.6%) in the amorolfine group respectively. The observed difference in increase of percentage of healthy surface after application of the acidifying solution was statistically significant (95% CI: 0.4; 12.1, p = 0.037) in comparison to the amorolfine group. Both treatments resulted in significant (p < 0.05) improvement after 168 days (vs. baseline) for nail dystrophy, discoloration, nail thickening, and healthy aspect but effects were more pronounced in the acetic acid group. Microbiological results and improved quality of life further confirmed clinical efficacy. Both treatments were well tolerated and appreciated for their properties and efficacy.

CONCLUSIONS: The present trial confirmed clinical performance of daily acidification of the nail, as reflected by 1) the superior increase of percentage of healthy nail surface when compared to amorolf-

Corresponding Author

Pedro-Antonio Regidor, MD; e-mail: pedro-antonio.regidor@exeltis.com

ine; 2) the overall improvement of other onychomycosis-related parameters; 3) the convenience and absence of significant side effects. These data indicate that acid/acid ester solutions can be a convenient, safe and equally effective alternative for the topical management of onychomycosis.

KEYWORDS
Onychomycosis, Nail acidification, 5% amorolfine nail lacquer.

INTRODUCTION

Onychomycosis is a common nail infection with a worldwide prevalence of 5%, but this value may vary in function of the studied area. Most common pathogens are dermatophytes, but also yeasts (e.g. *Candida albicans*), and non-dermatophyte molds[1]. Depending on the location and the route of pathogen penetration, four different types of onychomycosis have been characterized: 1) disto-lateral onychomycosis; 2) white superficial onychomycosis; 3) proximal subungual onychomycosis; 4) candidal onychomycosis. Disto-lateral subungual onychomycosis is the most common form and is usually caused by *Trichophyton rubrum*, which invades the nail bed and the underside of the nail plate[2-4].

Onychomycosis gradually destructs the nail by affecting the nail plate, the nail bed and the periungual tissue. Depending on the degree of infection, nail discoloration, thickening (onychogryphosis), degeneration (dystrophy), brittleness, and loosening (onycholysis) are observed[5]. Although the disease is not life-threatening, its morbidity may negatively impact patient's quality of life[6].

Efficient treatment is challenging because of the inherent slow growth of the nail and its composition as well as patient compliance. Also, comorbidity in risk groups (e.g. elderly, diabetic, immunosuppressed, or psoriasis patients) will further hamper treatment[7].

Early oral medication has been shown to be rather ineffective against specific forms of onychomycosis, in particular superficial onychomycosis and endonyx forms. Manufacturers have focused on the development of topical products, that affect dermatophytes through a physical, non-specific or a pharmacological mode of action, respectively[8]. Most of the topical products are lacquers that need to be removed with solvents on weekly basis, a fact that stands in the way of patient compliance.

A randomized, controlled, multicentre, open label trial was performed to assess the clinical efficacy against onychomycosis of an acetic acid/ethyl lactate brush-on solution (Excilor®, 0.96% acetic acid in ethyl lactate) *vs.* a medicated nail lacquer containing 5% amorolfine (Loceryl®). After penetration, the acid/ester solution acidify the nail and consequently block fungal spreading, hereby allowing the infected part of the nail to grow out[9-10]. The amorolfine nail lacquer elicits its action by destroying the fungal cell membrane[11].

The primary objective of the present study was to assess variation in the percentage of healthy surface of the great toenail after a treatment period of 168 days with both products, in combination with changes in microbiological findings at baseline and at the end of the treatment (KOH staining and fungal culture). Clinical diagnosis was performed by blinded investigators by using digital image analysis (contour tracing). Secondary objectives included evaluation of clinical efficacy against onychomycosis of the great toenail at distinct time points (day 14, 28, 56, and 112), microbiological efficacy of both products, product safety, impact on quality of life (QoL), and finally product efficacy, tolerance and acceptability by subject's self-assessment and medical exam.

PATIENTS AND METHODS

Trial set-up

This randomized, controlled, multicentre, comparative, investigator-blinded, open label study, was approved by the Ethics committee of the principal clinical trial centre (Military Hospital of Tunis, Tunisia) on December 16th 2014. The study was conducted in accordance with the principles of the Declaration of Helsinki 2013, Good Clinical Practice, and of the European Union Directive 2001/20/EC.

The entire study took place in two clinical trial facilities in Tunis (Tunisia), specialized in treatment of skin and nail disorders. Recruitment was performed by the principal investigator (dermatologist) of each trial centre and continued from January 16th (first patient, first visit) to November 16th 2015 (last patient, last visit).

Inclusion and exclusion criteria

Patients (>18 years) were included after confirmed diagnosis of superficial onychomycosis on at least one great toe nail or light to moderate disto-lateral onychomycosis (without matrix involvement, infected area being smaller than 2/3 of the nail surface). Potassium hydroxide (KOH) staining was used to confirm diagnosis [11]. Briefly, collected nail fragments were treated with 30% KOH solution and incubated for 5-10 min. Next, microscopic analysis

was performed to assess the presence of dermatophytes, which were identified by their hyphae. Only patients with positive staining were included. Fungal culture was performed on samples of KOH-positive subjects to further characterize dermatophyte infection via macroscopic and microscopic analysis. Yet, outcome of these fungal cultures did not restrict subject inclusion since false negative results regularly occur in clinically confirmed cases[12].

Beside positive diagnosis, patients must have stopped any systemic and/or topical antifungal treatment for at least 6 and 3 months, respectively, before inclusion. Finally, female subjects of childbearing potential should use an accepted contraceptive regimen at least 12 weeks prior to study start, during the study, and at least 1 month after the study end.

Exclusion criteria were: non-compliance with the protocol, enrolment in another clinical trial during the test period, pregnant (or planning to) or nursing women, known allergy to one of the ingredients of both products, patients suffering from serious or progressive diseases (uncontrolled diabetes, peripheral circulatory disease, HIV, psoriasis, lichen planus, immunosuppressive disorders), and patients with other skin diseases in the studied zone.

Informed consent, randomization, and baseline data

Each subject received oral and written information concerning the studied product, its nature, the duration and the conditions of the study. Written consent was obtained before any study-specific procedures were performed in accordance to the Helsinki declaration.

Following this informed consent, a patient screening number was assigned to each patient. A randomisation list was provided prior to the start of the study. A unique randomisation number attributed each included patient to one of the treatment groups, with an equal probability (n=56 in each product arm). Baseline demographic data were collected on gender, age, height, weight, blood pressure parameters, and medication use.

Blinding

Discernible differences in the product properties (e.g. different bottle, odour) and in the administration process allowed patients to recognize both trial products. Therefore, blinding and unbiased evaluation was guaranteed by making digitalized macro-photographs of the toenail, which were in turn analysed by two blinded evaluators. The detailed procedure is described below in chapter "Evaluation of clinical efficacy".

Study medication, dosage and administration

The acetic acid solution (Excilor®) was supplied in glass bottles (with brush applicator) by Oystershell Laboratories (Ghent, Belgium). This product consists of acetic acid (active ingredient), solvent (ethyl lactate), a penetration enhancer, a film former, water, preservatives, acetylated lanolin alcohols, glycerol, and biotin.

The amorolfine nail lacquer reference (Loceryl®; available in glass bottle) was provided be the principal investigator. This medicated nail lacquer contains 5% amorolfine (as amorolfine hydrochloride in ethanol, triacetin, butyl acetate, ethyl acetate and ammonium methacrylate polymer).

The acetic acid solution was applied twice daily with the brush, covering the complete (cleaned) nail and the underside of the nail rim. If new growth appeared, the nail was trimmed using a nail clipper.

Amorolfine was applied once a week with a reusable spatula (supplied with the product). Prior to use, the nail was filed and cleaned with isopropanol wipes.

Evaluation of clinical efficacy

Patients were treated with the acetic acid solution or amorolfine lacquer, respectively, for a period of 168 days. Onychomycosis evolution was evaluated at distinct time points: day (D) 14, D 28, D 56, D 112, and D 168, and compared to D 0 (baseline). The primary objectives were to assess variation in the % of healthy surface of the great toenail at the end of the study (D 168) when compared to baseline in both treatment groups. Diagnosis was performed using digital image analysis. Briefly, at each time point, two macro-photographs (top and front) were made of the great toenail, placed beside a piece of graph paper to allow determination of the exact size of the nail during analysis (contour tracing). Consequently, all pictures were digitalised and recorded on the computer. Image analysis of the top picture was performed with Adobe Photoshop software[13]. For each photograph, a blinded dermatologist traced the healthy surface. Next, a second evaluator, also blinded, determined the percentage of healthy surface and assigned the following scores: 0 = 100% healthy surface, 1 = more than 66.6% healthy surface, 2 = 33.3-66% healthy surface, and 4 = less than 33.3% healthy surface.

Secondary objectives implied evaluation of the following parameters:

a) Clinical efficacy against onychomycosis of the great toenail at D 14, D 28, D 56, and D 112;
b) Microbiological efficacy of the product (KOH staining method);
c) Product safety;
d) Impact on the quality of life (QoL) of the subjects;
e) Product efficacy, tolerance and acceptability by subject's self-assessment.

At D 14, D 28, D 56, D 112, and D 168, the following parameters have been scored to assess onychomycosis evolution:

a) Onycholysis;
b) Nail dystrophy;
c) Nail discoloration;
d) Nail thickening.

The following scores were assigned: 0 = none, 1 = very slight, 2 = slight, 3 = moderate, 4 = severe.

All patients evaluated the efficacy and acceptability of the treatment regime by answering a questionnaire at each visit. In addition, at D 0 (baseline), D 56, D 112 and D 168, patients answered a validated questionnaire (NailQoL) to assess the impact of onychomycosis on their quality of life[14].

Safety evaluation

At each visit, the local tolerance (scored as "bad tolerance", "moderate tolerance", "good tolerance", and "very good tolerance") and the global tolerance (collection of all adverse events and subjective signs) were evaluated. In addition, all patients were asked to report adverse events into a logbook. Study staff investigated all adverse events and determined the relationship to the use of each product.

Statistical Analysis

Clinical efficacy was evaluated in the intention-to-treat (ITT) population, whereas safety and tolerability parameters were assessed in the "safety" population. Briefly, continuous data were summarized by their mean, standard deviation (SD), median, minimum and maximum. Categorical data have been summarized by frequencies and percentages.

Mean absolute changes in % healthy surface from baseline (D0) at D 168 between both products were compared with an independent *t*-test after having verified the assumptions of normality (QQ-plot) of the differences. Changes in % healthy surface from baseline in function of treatment duration were studied in more detail using linear mixed-effects model with fixed effects for time and treatment and random effect for subjects, after having verified normality and homoscedasticity of the residuals (QQ-plot and residuals *vs.* fitted values).

Mean absolute changes in global score from baseline (D0) at D168 between both products were compared with an independent t-test after having verified the assumptions of normality (QQ-plot) of the differences.

To compare changes in nail dystrophy, discoloration, and nail thickening between baseline and D168, five categories were reduced to two categories (None to slight *vs.* moderate to severe). The same was done for healthy aspect of the nail (totally and quite healthy *vs.* moderate to not healthy at all). The McNemar test for paired data was used to test if there was a change in nail dystrophy, discoloration, and nail thickening between baseline and D168.

All descriptive and statistical analyses were performed in R version 3.3.1. (R development core team, 2016). A p-value < 0.05 was considered as statistical significant. No imputation of missing data is performed. The amount of missing data is presented in the tables wherever appropriate.

Baseline data

In total, 112 subjects were randomized into the study, with 56 persons in each treatment group. Seven subjects (n=2 in the acetic acid group and n=5 in the amorolfine group) did not complete the study (withdrawal of consent or lost to follow-up), whereas clinical data of D 168 from 3 subjects were not available. For this reason, 10 subjects were excluded from the analysis, yielding a total of 102 subjects (n=53, acetic acid; n=49, amorolfine). For safety and tolerability analysis, 108 subjects (n=54, acetic acid; n=54, amorolfine) were included into the safety population. A summary of demographic characteristics is presented in Table 1.

Prior to product application (D 0), no significant differences were found between both treatment groups for average healthy surface (p=0.1353), secondary clinical parameters (different p-values; not shown), and average NailQoL score (p=0.3920).

Table I. Demographic characteristics.

	Test product	Reference
Age (average ± standard deviation (SD)	46.5 ± 13.2 yrs.	46.8 ± 12.8 yrs.
Minimum - median - maximum	20-47 - 83	20-48.5 - 77
Sex		
– Male, n (%)	22 (39.3)	20 (35.7)
– Female, n (%)	34 (60.7)	36 (64.3)
% healthy surface (average ± SD)	64.0 ± 13.3%	66.8 ± 9.8 %
NailQoL Score (average ± SD)	57.7 ± 10.3	56.0 ± 12.9
KOH staining	100%	100%
Fungal culture	65% positive	68% positive
Genus of fungi		
– *Trichophyton rubrum*	75.0%	77.8%
– *Trichophyton interdigitalae*	19.4%	11.1%
– *Trichophyton mentagrophytes*	0%	2.8%
– *Aspergillus niger*	5.6%	5.6%
– *Aspergillus fusarium*	0%	2.8%

Direct detection of fungal infection with the KOH staining method was positive for all subjects in both treatment groups. Consequent fungal culture was positive for a majority of the subjects, with *Trichophyton (T.) rubrum* being the most common pathogen (75 and 78% for the acetic acid solution and amorolfine group, respectively). Other dermatophyte fungi were also detected, including *T. interdigitalae* (both groups), and *T. mentagrophytes* (reference group only). Infections with non-dermatophytic pathogens (*Aspergillus niger* and *Aspergillus fusarium*) were very limited.

RESULTS

Efficacy evaluation

Primary efficacy: change in percentage of healthy surface

The efficacy of both treatments was compared in terms of percentage of healthy surface (Table 2).

The percentage of healthy surface between baseline and D 168 increased with 11.4% (SD=17.0) in the acetic acid group and 5.1% (SD=12.6) in the amorolfine group, respectively. The observed difference in increase of percentage of healthy surface was statistically significant (95% CI: 0.4; 12.1, p = 0.037).

The percentage of healthy surface after 14, 28, 56, 112 and 168 days of treatment was further compared between both treatment groups with a generalized linear mixed-effects model. This model confirmed a significant increase in % of healthy surface in function of treatment duration (p<0.001). Furthermore, this improvement was significantly higher after 112 (7.3%, p = 0.006) and 168 days (6.2%, p = 0.015) of treatment with the acetic acid solution *vs.* the amorolfine nail lacquer.

Secondary efficacy criteria

Onychomycosis evolution

The proportion of subjects with an improvement or success increased from 8.9% after 14 days ((53.6% (D 28), 76.8% (D 56), 87.3% (D 112)) to 92.6% after 168 days of treatment with the acetic acid solution. Improvement or success was also observed with reference but this was less pronounced and remained more or less the same between days 56 (62.3%) and 168 (56.9%). After 112 and 168 days of treatment, significantly (p=0.035 and p<0.001, respectively) more subjects from the test product group showed improvement or success in comparison to the reference group.

Onycholysis

No important changes in onycholysis were observed over treatment time and between both substances.

Dystrophy

At baseline, moderate to severe nail dystrophy was observed in 50% of the subjects (acetic acid group: 51.8% (29/56) and in the amorolfine group: 48.2% (27/56). Generally, nail dystrophy improved over treatment time, but the improvement was more pronounced in the acetic acid group. After 168 days of treatment, moderate to severe nail dystrophy was observed for 9.3% (5/54) of the subjects in the acetic acid group in comparison to 29.4% (15/51) in the amorolfine group. Of note, both substances resulted in a significant improvement of nail dystrophy (McNemar p < 0.001 and p = 0.034, respectively) between baseline and D168.

Discoloration

At baseline, moderate to severe discoloration was observed in 92.0% of the subjects (acetic acid group: 94.6% (53/56) and in the amorolfine group: 89.3% (50/56). Generally, nail discoloration improved over treatment time, but the improvement was more pronounced in the acetic acid group. After 168 days of treatment, moderate to severe discoloration was observed for 9.3% (5/53) of the subjects in the acetic acid group in comparison to 43.1% (22/51) for the amorolfine group. Both substances resulted in a significant improvement of discoloration (McNemar, p < 0.001 and p < 0.001, respectively) between baseline and D168.

Nail thickening

At baseline, moderate to severe nail thickening was observed in 87.5% of the subjects (acetic acid group: 82.1% (46/56) and in the amorolfine group: 92.9% (52/56). Generally, nail thickening improved over treatment time; however, the improvement was more pronounced in the acetic acid group. After 168 days of treatment, moderate to severe nail thickening

Table II. Efficacy of treatment with an acid solution *vs.* 5 % amorolfine: summary statistics for the % of healthy surface (mean ± SD) (number of subjects in brackets).

Treatment	D0	D14	D28	D56	D112	D168
Acid solution	64.0 ± 13.3 (55)	67.6 ± 15.1 (50)	69.1 ± 14.5 (48)	70.5 ± 15.7 (53)	71.6 ± 15.2 (49)	74.8 ± 12.3 (54)
Amorolfine	66.8 ± 9.8 (55)	68.0 ± 12.7 (52)	71.7 ± 12.1 (47)	70.6 ± 13.0 (47)	67.8 ± 13.0 (46)	72.3 ± 12.9 (49-

was observed for 13.0% (7/54) of the subjects in the acetic acid group in comparison to 35.3% (18/51) for the amorolfine group. Both substances resulted in a significant improvement of nail dystrophy (McNemar $p < 0.001$ and $p < 0.001$, respectively) between baseline and D168.

Healthy aspect of nail

At baseline, quite to totally healthy was observed in 15.2% of the subjects (acetic acid group: 14.3% (8/56) and amorolfine group: 16.1% (9/56). Generally, the healthy aspect of the nail improved over treatment time, but this improvement was more pronounced in the acetic acid product group. After 168 days of treatment, quite to totally healthy was observed for 64.8% (35/54) of the subjects in the acetic acid group in comparison to 37.2% (19/51) for the amorolfine group. Both substances resulted in a significant improvement of nail dystrophy (McNemar $p < 0.001$ and $p = 0.010$, respectively) between baseline and D168.

Secondary efficacy criterion: microbiological evaluation

After 168 days, only 37% in the acetic acid group and 38% of the subjects in the amorolfine group remained KOH positive. Results of fungal culture demonstrated a decrease of 51% (66% to 15%; acetic acid group) and 56% (68% to 12%; amorolfine group), respectively, at day 168. These differences were not statistically significant.

Secondary efficacy criterion: evaluation of subjects' quality of life

Efficacy of both products on the quality of life (QoL) of the subjects was evaluated with the validated NailQoL questionnaire[15] at the start of the study, and 56, 112 and 168 days after start of the treatment, respectively. Summary statistics are provided in Table 3.

A NailQoL global score of 0 corresponds to a quality of life never altered by onychomycosis, whereas a score of 100 corresponds to a quality of life that is always affected by onychomycosis. Both treatments resulted in a reduction of the NailQoL global score in function of duration of the therapy, indicating an improvement of subject's quality of life. After 168 days of treatment with the acetic acid solution, mean NailQoL score decreased with 38.9 units compared to baseline. For subjects treated with amorolfine, mean NailQoL score decreased with 29.7 units. This improvement in subject's quality of life after 168 days was significantly higher for subjects treated with the acetic acid solution, showing an improvement of on average 9.2 units (95% CI: 3.1 to 15.2, p=0.003) when compared to amorolfine.

Safety evaluation

Local tolerance of the treatment was assessed by the investigator via clinical evaluation and subject interrogatory at each visit during the trial. Overall, both treatments were very well tolerated with a score = 3 during each visit, with the exception of one subject from the reference group who received a score of 2 (good tolerance) during one visit (D 14).

DISCUSSION

Fungal infections are reported to cause 23% of foot diseases and 50% of nail conditions in people seen by dermatologists, but are less common in the general population, affecting 3% to 5% of people[16]. The prevalence varies among populations, which may be due to differences in screening techniques. In one large European project (13,695 people with a range of foot conditions), 35% had a fungal infection diagnosed by microscopy/culture[17]. One prospective study in Spain (1000 adults aged > 20 years) reported a prevalence of fungal toenail infection as 2.7% (infection defined as clinically abnormal nails with positive microscopy and culture)[18]. In Denmark, one study (5755 adults aged > 18 years) reported the prevalence of fungal toenail infection as 4.0% (determined by positive fungal cultures)[19]. The incidence of mycotic nail infections may have increased over the past few years, perhaps because of increasing use of systemic antibiotics, immunosuppressive treatment, more advanced surgical techniques, and the increasing incidence of HIV infection[20].

During recent years, different topical products have been put on the market for the treatment of onychomycosis. They are used either alone or in combination with systemic treatments, resulting in higher cure rates. For topical treatment, both medicated nail solutions and medical devices with a

Table III. Summary statistics for the evaluation of subject's quality of life (NailQoL global score) by treatment and in function of time (number of subjects in brackets).

Treatment	D0	D56	D112	D168
Acid solution	57.7 ± 10.3 (56)	29.8 ± 15.5 (56)	24.0 ± 14.1 (55)	18.7 ± 16.3 (54)
Amorolfine	56.0 ± 12.9 (56)	32.3 ± 15.8 (53)	26.9 ± 16.4 (51)	26.4 ± 15.0 (51)

physical mode of action are commercially available. In the present study, the acetic acid-based nail solution, which inhibits fungal growth by acidification of the nail environment, was compared to a nail lacquer containing 5% amorolfine[21-22]. Amorolfine is a morpholine antifungal drug, which disrupts the fungal cell membrane[10].

All subjects were diagnosed with either superficial onychomycosis or light to moderate disto-lateral onychomycosis (no affection of matrix; involvement < 2/3 of the tablet) on at least one great toenail. Fungal infection was further confirmed using the KOH staining method[11].

Both women and men were included, with a higher proportion of women. Average age was 46.5 ± 13.2 years and 46.8 ± 12.8 years in the acetic acid and amorolfine group, respectively. At baseline (D 0), both treatment arms were homogeneous for all studied parameters.

At the end of the study (D 168), 7 subjects (n=2, test product; n=5, reference) did not complete the study (withdrawal of consent or lost to follow-up), whereas clinical data of D 168 from 3 subjects were not available. For this reason, these subjects were excluded from the data analyses, resulting in a final number of 102 patients. Safety and tolerability analysis was performed in the safety population, consisting of 108 subjects (n=54 for both groups).

The primary objective of this study implied evaluation of the effect of both treatments on the evolution in % of healthy surface between baseline and D 168. For the acetic acid product, an increase of 11.4% was observed, whereas treatment with amorolfine resulted in an increase of 5.1%. The difference in increase was significantly different (95% CI: 0.4; 12.1, $p = 0.037$). Clinical performance of the acetic acid solution was further confirmed by the number of patients showing onychomycosis improvement or success (completely cured) at the end of the study: 92.6% (acetic acid) *vs.* 56.9% (amorolfine). Again, the difference between both treatment arms was significant ($p < 0.001$).

Evaluation of other onychomycosis-related parameters demonstrated that the effect of the acetic acid solution (when compared to amorolfine) was more pronounced for nail dystrophy, discoloration, nail thickening, and healthy aspect of the nail. For all parameters, a significant improvement when compared to baseline was shown for both treatments.

Clinical efficacy was further reflected by the improvement in patient's quality of life, as evaluated using a validated questionnaire (NailQoL)[14]. This was observed in both treatment arms but at study end, the effect in the acetic acid group was significantly more pronounced.

Finally, both treatments were well tolerated, hereby confirming product safety.

The mode of action of the acid solution, which contains acetic acid and the acidic ester ethyl lactate, relies on acidification of the nail. Following application, acid penetration and consequent pH decrease of the nail environment will inhibit acid-sensitive keratolytic enzymes, which are essential for dermatophyte nail penetration[9, 23-25]. In turn, fungal growth inhibition allows the infected part to grow out *in vivo*, without further fungal spreading.

Susceptibility of dermatophytes towards acids has been demonstrated in independent experiments and literature reports. Results of a "minimum inhibitory concentration" assay confirmed fungal growth inhibition following exposure to different organic acids, including acetic acid[8]. Furthermore, in a validated bovine hoof assay, both acetic acid solution and the amorolfine product were able to penetrate the nail and to inhibit *Trichophyton mentagrophytes* growth[9]. These *in vitro* data are further confirmed by the results of the present clinical trial.

CONCLUSIONS

The tested acetic acid solution is an efficient and safe treatment for mild to moderate cases of onychomycosis. At study end, the % of healthy surface of the nail was significantly more increased when compared to Loceryl®. Clinical performance of the test product was further confirmed by: 1) the significantly higher number of patients with onychomycosis improvement or success (92.6% test product *vs.* 56.9% reference); 2) the more pronounced positive evolution of onychomycosis-related parameters in function of time; 3) the positive impact on quality of life of the patients; 4) confirmed safety. The present clinical data confirm that the tested medical device is a safe and an adequate alternative for medicated nail lacquers for the treatment of superficial onychomycosis or light to moderate disto-lateral onychomycosis.

Conflicts of interest:
Funder of the study was Oystershell Laboratories, who is also the manufacturer of the test product and employer of the co-authors Frank Eertmans and Bart Rossel. The sponsor was involved in the study planning as well as in the decision to publish, but was not involved in data collection, data analysis and data interpretation. Oystershell Laboratories engaged the CRO Dermscan (Laboratoires Dermscan, Villeurbanne, France) to independently design and perform the study. Co-author Professor Néjib Doss was the principle investigator of the study and responsible for patient investigation / recruitment and data collection. Statistical analyses were performed by an independent, external consultant, Els Adriaens (Adriaens Consulting, Bellegem, Belgium). Pedro-Antonio Regidor is Medical Director Western Europe and Germany Exeltis.

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