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Efficacy of a medical grade honey formulation (L-Mesitran) in comparison with fluconazole in the treatment of women with recurrent vulvovaginal candidiasis: protocol for a randomised controlled trial (HONEY STUDY)

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ABSTRACT

Introduction Recurrent vulvovaginal candidiasis (RVC) affects up to 9% of women worldwide. This amount is expected to increase due to lifestyle changes, increased fungal resistance and biofilm formation. Treatment options are limited and in 57% of the cases, relapses occur within 12 months after starting fluconazole therapy (golden standard). The pathogenesis of RVC is multifactorial and includes fungal biology, the vaginal microenvironment and the immune system. Fluconazole is antimicrobial and effective in inducing short-term remission but a long-term cure is hard to achieve. Medical grade honey (MGH) has antimicrobial, protective, antioxidative and immunomodulatory activity and may therefore be a good alternative treatment. This study aims to investigate the clinical cure rate and long-term efficacy of MGH compared with fluconazole in patients with RVC.

Methods and analysis This study is a multicentre, randomised controlled trial (Maastricht University Medical Centre+ and Zuyderland Medical Centre). A total of 252 eligible women will be randomly assigned to the fluconazole group (control) or the MGH group (L-Mesitran, treatment). The primary objective is to investigate the mycological cure rate after 1 month assessed through a vaginal culture. Secondary objectives are the clinical cure rate after symptoms, the prophylactic activity after 6 months of maintenance therapy and the number of relapses within 12 months. Moreover, information about side effects, discomfort and quality of life will be collected with the use of questionnaires.

Strenghts and limitations of this study

⇒ First large randomised controlled trial with 252 women with recurrent vulvovaginal candidiasis comparing medical grade honey to standard of care.
⇒ Multiple outcome measures, mycological and clinical cure rates, symptoms, quality of life, costs, etc.
⇒ Relative long follow-up (12 months) to determine the long-term cure rate of both treatments.
⇒ The main limitation of this study is possible loss to follow-up due to the relatively long follow-up period.
⇒ Blinding is not possible due to the unavailability of proper placebos and administration routes.

INTRODUCTION

Approximately 75% of women develop vulvovaginal candidiasis (VVC) at least once in their life.¹ Current treatment regimens for VVC are not optimally effective, which contributes to the relatively high recurrence rate. The definition of recurrent VVC (RVVC) is having three or more symptom-atic episodes of VVC and at least one positive vaginal culture with complete relief of problems in between episodes.²

Women between 25 and 34 years of age have the highest prevalence of recurrent infections (9%).³ In 2016, 138 million women worldwide were suffering from RVC. It is estimated that this number will increase to 158 million women in 2030.³ Literature shows that 57.1% of patients with RVVC develop a recurrence of problems within a year after treatment with fluconazole, the current golden standard for the treatment of VVC.³

The pathogenesis of RVC is multifactorial. Four key factors determine the
progression and development of RVVC: the presence of Candida, the population of Lactobacilli, the microenvironment, and host-related factors. A disbalance in any of these factors may induce RVVC. 

Host-related factors that contribute to the increased rate of VVC development and recurrence of the disease are misuse of antibiotic therapy, an ageing and more sexually active population using hormone replacement therapy, and the increasing use of sodium-glucose cotransporter-2 inhibitors as a treatment for diabetes mellitus. 

Remarkable is that therapy and recurrence of the disease are misuse of antibiotic that contribute to the increased rate of VVC development. 

Fluconazole is antimicrobial and thereby effective in inducing remission, but long-term cure is hard to achieve and maintain. 

Fluconazole will become more often inadequate in the future due to increasing resistance to fluconazole, the rise of non-albicans candida (NAC) species which are less susceptible to azoles, and the existence of biofilms with a 1000-fold higher resistance profile. 

Currently no treatment targets Candida biofilm eradication.

Medical grade honey (MGH) has antimicrobial, protective, antioxidative, anti-biofilm and immunomodulatory activity and may therefore be a promising alternative treatment, especially in long-term remission. 

In vitro studies and clinical studies presented medical honey as a good alternative treatment for RVVC. In these in vitro studies, Candida species of patients with RVVC were isolated, and minimal inhibitory concentrations and minimal fungicidal concentrations were determined. Eleven in vitro studies performed between 2005 and 2021 showed that honey was effective against Candida species, against isolates who are resistant to itraconazole and/or fluconazole, and against Candida biofilms. 

Eight clinical studies performed between 2012 and 2021 investigated the effect of honey on (R)VVC in comparison to antimycotics. 

Five studies demonstrate significantly better results with honey in relieving symptoms of RVVC, two studies show comparable results and in one study antimycotics present better results. MGH has the potential to resolve candidiasis and decrease the number of relapses. Since there is no effective alternative treatment to fluconazole, this study may have a significant impact and improve the quality of life of women suffering from RVVC.

The aim of this randomised controlled trial (RCT) is to compare fluconazole with MGH in women with RVVC regarding its clinical efficacy and prophylactic activity.

METHODS AND ANALYSIS

Objectives and outcome measures

The primary objective is to compare the mycological cure rate after 1 month of therapy between the control (fluconazole) and treatment (L-Mesitran, an MGH-based formulation) groups via a vaginal culture. The vaginal cultures will be analysed in the Maastricht University Medical Centre+ (MUMC+) laboratory to determine the presence or absence of Candida.

Secondary objectives are to investigate the prophylactic activity after 6 months maintenance therapy and the number of relapses within 12 months will be analysed for long-term efficacy via a vaginal culture. In addition, the clinical cure rate of MGH and fluconazole on the symptoms, including redness, irritation, itching, dysuria, dyspareunia and vaginal discharge will be investigated after 1, 6, 9 and 12 months of therapy. These parameters will be evaluated using questionnaires, and scored as 0 (absent), 1 (mild), 2 (moderate) and 3 (severe).

Patient and public involvement

No patient involved.

Study design, participants

The study will be a multicentre RCT performed at the Maastricht University Medical Centre and the Zuyderland Medical Centre in the Netherlands.

Patients with RVVC defined as at least one positive Candida culture and at least three episodes of symptoms in 1 year who meet the inclusion criteria will be informed about the study. Women 18 years and older of age, native Dutch speaker, with a diagnosis of RVVC as earlier defined and having a positive Candida culture at the time of consultation and who give written and oral informed consent are eligible to participate in this study. Exclusion criteria are having a mixed vaginal infection, having taken systemic or topical antifungal medication during the last 2 weeks prior to inclusion, being pregnant or having the intention to become pregnant during the study period, women giving breast feeding, having an allergy to fluconazole or honey or having a Candida with resistance to fluconazole. The investigator can decide to withdraw a subject from the study for urgent medical reasons. The recruitment has started in August 2022 and is ongoing.

Recruitment and consent

All patients with problems of RVVC at time of consultation will be informed about the study by the treating gynaecologist. Eligible women who fulfill the inclusion criteria will be identified and counselled by the research coordinator or staff of the participating centres. The patient will be asked if she may be contacted at a later stage by the researcher/research nurse regarding this study. Before including this study, the research coordinator and/or the staff will explain to potential participants the aims, methods, reasonably anticipated benefits and potential hazards of the study. They will be informed that their participation is voluntary and that they may withdraw consent to participate at any time during this study. They will be informed that choosing not to participate will not affect their care. After giving sufficient information, written informed consent must be obtained. If the patient does not want to participate or not meets all
criteria, she will not be included and randomised to the study. See figure 1.

**Randomisation, blinding and treatment allocation**

Randomisation will be performed using a data management programme of the participating hospitals (Castor) after a patient has been included. Patients will be randomised by block randomisation using random permuted block sizes stratified by age (categories: 18–35 years, 35–50 years and 50 years and older). The randomisation code will be strictly followed, and we do not foresee any reason to break the randomisation code. Unfortunately, it is impossible to blind the patients for the treatment, as one of the treatments must be taken orally and the other is a honey formulation that needs to be applied intravaginally. To guarantee an objective analysis, we use validated and widely accepted outcome measures like vaginal cultures and validated questionnaires.

**Collection of data**

**Questionnaire**

The electronic online questionnaires will be sent by Castor. Patient characteristics (age, comorbidities, medication use, use of anticonceptive, number of sexual partners during the last year) will be copied in Castor from the electronic patient record. Patients will receive the

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**Figure 1** Flowchart of the study. MGH, medical grade honey.
questionnaire by email after randomisation and the first questionnaire needs to be completed before treatment starts. When the patient does not respond after 7 days, Castor will send a reminder to fill in the questionnaire. The questionnaire contains the 36-Item Short Form Survey (SF-36) as a measure of the quality of life and some specific questions to obtain a more specified insight into the disease and the treatment. Moreover, information about side effects, discomfort, quality of life and therapy compliance will be collected and compared. After 1, 6, 9 and 12 months the patients have to fill in the online questionnaire. This questionnaire contains questions about relevant medical history, patient characteristics, symptoms, quality of life and treatment.

Vaginal culture
The patient has to take a self-collected vaginal swab at 1, 6 and 12 months after the start of treatment. Patients will receive a reminder together with the questionnaire to collect the swabs and send them. The vaginal cultures will be analysed in the MUMC+ laboratory to determine the presence (positive) or absence (negative) of Candida.

Handling and storage of data and documents
After randomisation patients will receive a unique study number which is used throughout the trial. The coding is performed as follows: HONEYM001-HONEYM252 for patients from MUMC+ and HONEYZ001-HONEYZ252 for patients from Zuyderland Medical Centre. This unique study number (like HONEYM001) is linked to the identification in the electronic patient database (EPD) of the patient. A list is made in excel which contains an overview of the assigned patients (key file). This file contains all important information that is needed to guarantee the privacy of the patients, and thus holds the unique study numbers coupled to the patient identification codes (EPD) and information to which group they are assigned. Only the principal investigator and the coordinating investigator in each hospital have access to this key. This file is saved in a secure folder, intended specifically for this project, on secure drives which can only be accessed by the involved investigators using a password. The signed informed consent forms are stored in a physical locker that is only accessible by the principal investigator and coordinating investigator. The patient gives the researcher informed consent to access relevant data from the treating physician to obtain results regarding the culture, diagnosis, progression, issues, medication and gynaecological medical history.

Patients will stay pseudonymised for the investigators and their privacy is protected. At multiple time points, vaginal swabs are taken by the patient. These self-sampling kits will be labelled with the patient code HONEYM001-HONEYM252 or HONEYZ001-HONEYZ252 and provided at the start of the study, at the same time as the medication. After the MUMC+ laboratory has performed the mycological tests, the samples will be destructed in the same way as all other patient material in a biohazard container within 1 week after testing. The data of the study will be stored for 15 years after the study has been completed, in accordance to the Good Clinical Practice (GCP) and the ‘EU General Data Protection Regulation (GDPR, in Dutch AVG)’ guidelines, and the ‘Dutch Act on Implementation of the GDPR (in Dutch: UAVG)’. With the approval of the patients, the contact information of patients and the research data will be stored for a possible follow-up study, this will also be stored for 15 years after the study has been completed. The data cannot leave the European Union, only when the article is published it will be accessible to the public but data is anonymised and not traceable to patients.

Interventions
Fluconazol group
The patients from the fluconazole group will receive a prescription for 28 fluconazole oral capsules in total. The dose of fluconazole is 150 mg per capsule. The patients will receive in the first week three capsules of fluconazole, respectively, one capsule on day 1, one capsule on day 4 and one capsule on day 7 followed by one capsule of fluconazole per week for a duration of 6 months every week on the same day.

MGH group (L-Mesitran Soft)
The product L-Mesitran consists of 40% MGH and is supplemented with other ingredients, such as the antioxidants vitamins C and E, lanolin, PEG4000 and propylene glycol, to ease application and enhance its beneficial anti-microbial and wound healing activities as supported by the literature. In addition, this product is registered as a medical device and follows strict criteria to ensure its quality, safety and efficacy. The patients from the L-Mesitran Soft group receive six tubes of 50 g L-Mesitran Soft with 52 applicators of 5 g. For the first month (three tubes in total), as a treatment for active Candida, patients need to apply the complete content of one applicator every day, preferably at night just before going to sleep. As prophylaxis to prevent a new RVVC episode, patients need to apply one applicator per week in the same way for a duration of 5 months (requiring three tubes) (6 months of total treatment time).

Statistics
Sample size calculation
The sample size calculation is based on a similar study in which the effect of honey was investigated for the treatment of VVC. They reported a clinical cure rate of 86.6% in the honey group and 40% in the fluconazole group. Since the mycological cure rate was 76.6% in the honey group and 43.4% in the control group, we decided to use the mycological cure from the control group in our calculation. We think that a smaller difference than previously observed, that is, an absolute difference of 20% between groups instead of over 30%, can be considered clinically meaningful. To be able to have 80% power to detect a difference of 20% assuming the success rate in
the control group is 43.4%, we need to include at least 94 patients per group when using an alpha of 5% for testing. To compensate for a dropout rate of up to 25% we need to include 126 patients per group or 252 in total. The calculation has been performed in R using the formula for the difference in proportions from Chow et al.\textsuperscript{34}

**Data analysis**

Baseline characteristics will be stratified by treatment allocation and described using mean and SD, median and first and third quartile and count and proportion. In case of missing outcomes in over 5% of patients, we will use multiple imputation to prevent a loss of statistical power and to reduce the likelihood of biased treatment effects. The number of imputations will be set to the percentage of incomplete patients. We will use fully conditional specification and the values to be imputed will be drawn using predictive mean matching. The analyses will be performed blinded for the treatment allocation. The study will be unblinded after analyses of the primary and secondary outcome measures. As an exploratory analysis, we will perform subgroup analyses of the primary outcome for patients that received previous treatments and for patients without previous treatments.

The outcome of the study is the percentage of patients with negative culture, based on the results of the vaginal culture. This is measured at 1, 6 and 12 months. The primary endpoint is the percentage at 1 month. Percentages will be estimated including their 95% CI stratified by group. Between-group differences will be tested using logistic regression analysis adjusted for the randomisation stratification variable (ie, age).

All binary secondary outcomes (ie, vaginal cultures (6 and 12 months) and clinical symptoms as measured by the questionnaires (occurrence of redness, irritation, itching, dysuria, dyspareunia, vaginal discharge, side effects and discomfort) after 6, 9 and 12 months of therapy) will be described as percentage and 95% CI and tested between groups using logistic regression analysis.

Long-term efficacy, defined as the number of relapses within 12 months, will be compared between groups using Poisson regression or, depending on the distribution, an alternative (eg, linear regression analysis). Quality of life will be quantified using the mean and 95% CI and compared between groups using linear regression analysis. Treatment compliance will be described as the percentage (95% CI) of patients who are not compliant and the median number of days treatment was taken, including the first and third quartile. All analyses will be performed according to the intention to treat principle. An alpha of 0.05 will be used for testing.

**DISCUSSION**

The population of women with RVVC is estimated to increase over the coming years.\textsuperscript{1} The most commonly used treatment for RVVC is fluconazole, which seems to become inadequate in the future due to increasing resistance to fluconazole, the rise of NAC species, and the existence of biofilms.\textsuperscript{3,5,6,10,35} MGH might be a promising treatment in VVC and RVVC. In contrast to fluconazole, MGH is expected to have multiple beneficial mechanisms. In addition to the antifungal activity on Candida, MGH can likely also eliminate antifungal-resistant Candida species, including NAC species and eradicate biofilms.\textsuperscript{15} Multiple in vitro studies demonstrated that different honey types inhibit the formation of Candida biofilms and can disrupt established biofilms.\textsuperscript{15} Moreover, MGH can modulate the microenvironment through its anti-inflammatory and antioxidative activity.\textsuperscript{11,36} In vitro studies and clinical studies demonstrated honey as a promising alternative therapy for RVVC.\textsuperscript{13} More research is needed to investigate the exact clinical efficacy and the long-term cure rate of MGH; as proposed, this will be further investigated in an RCT. This will be the first multicentre RCT that compares fluconazole with MGH in women with RVVC and may have a great impact on women suffering from RVVC.

**Ethics and dissemination**

Ethical approval from the Medical Ethics Review Committee (METC) of academic hospital Maastricht/University Maastricht (NL 73974.068.21 on 8 February 2022). Additional approval was obtained from the Ethics Committee of the Zuyderland Medical Centre Heerlen has been obtained (Z2021141 on 4 March 2022). The protocol of the Honey study is registered at ClinicalTrials.gov on 10 May 2022. In case of important protocol modifications, the METC and Ethics Committee of the Zuyderland Medical Centre will be informed. The study will be conducted according to the principles of the Declaration of Helsinki; version 2013 and in accordance with the Medical Research Involving Human Subjects Act. The study will be monitored by an independent data monitoring committee, Clinical Trial Center Maastricht. There is insurance for the participants which is in accordance with the legal requirements in the Netherlands. This insurance provides cover for damage to research subjects through injury or death caused by the study. The results of this RCT will be disseminated through presentations at international conferences and in peer-reviewed journals in accordance with academic standards.

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**Contributors**

SLJMr, CMJGL, MMLHW and NC were responsible for the development of the trial. SJK performed the statistical calculations of this study. SLJMr, CMJGL and MMLHW are responsible for the management and logistical supervision of the study. CMJGL, MMLHW and NC were responsible for the management and logistical supervision of the study.
aspects of the trial. All authors read and approved the final paper. SJJMVR is the coordinating investigator of this randomised control trial, CMJGL is the principal investigator of Maastricht University Medical Centre+ and MMLHW is the principal investigator of Zuyderland Medical Centre.

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Competing interests
The principal and coordinating investigators have no competing interests. NC is employed by Triticum Exploitatie BV. Triticum Exploitatie BV is the manufacturer of the medical grade honey (MGH)-based product that will be used in the study and is a co-funder of the study. As an expert on MGH, NC helped with the concept and development of the study, but he did not have any ultimate authorisation over the study design, collection management, analysis and interpretation of the data; writing of the report; or the decision to submit the report for publication.

Patient and public involvement
Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication
Not applicable.

Provenance and peer review
Not commissioned; externally peer reviewed.

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