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A proof of concept pilot trial of probiotics in symptomatic oral lichen planus (CABRIO)

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Abstract

Objective: To preliminary evaluate the clinical effects of probiotics in individuals with symptomatic oral lichen planus and the possible mechanisms of action.

Subjects and Methods: A group of 30 individuals with symptomatic oral lichen planus were recruited in a randomised double-blind parallel group controlled (1:1) proof-of-concept pilot trial of probiotic VSL#3 vs placebo. Efficacy outcomes included changes in pain numeric rating scale, oral disease severity score and the chronic oral mucosal disease questionnaire. Adverse effects, home diary and withdrawals were assessed as feasibility outcomes. Mechanistic outcomes included changes in salivary and serum levels of CXCL10 and IFN-γ and in oral microbial composition.

Results: The probiotic VSL#3 was safe and well tolerated. We observed no statistically significant change in pain, disease activity, quality of life, serum/salivary CXCL10 or oral microbial composition with respect to placebo. Salivary IFN-γ levels demonstrate a trend for a reduced level in the active group ($p = 0.082$) after 30 days of probiotic consumption.

Conclusions: The present proof-of-concept study provides some weak not convincing indication of biological and clinical effects of probiotic VSL#3 in individuals with painful oral lichen planus. Further research in this field is needed, with the current study providing useful information to the design of future clinical trials.

KEYWORDS
oral lichen planus, probiotic, the clinical and biological effects of the use of probiotic in patients with oral lichen planus, VSL#3

1 | INTRODUCTION

Oral lichen planus (OLP) is a common chronic inflammatory disease of the oral mucosa, with a global prevalence of 1.01% (Gonzalez-Moles et al., 2020). The pathogenesis of OLP is believed to involve a dysregulation of the immune system, (Jontell and Holmstrup, 2015; Zucoloto et al., 2019) including presentation of an unknown antigen, T lymphocyte activation and migration, production of pro-inflammatory cytokines and chemokines resulting in sub-epithelial inflammatory infiltrate, keratinocytes damage and disruption of epithelial homeostasis. (Ke et al., 2017; Lu et al., 2015; Marshall et al., 2017) Available evidence suggests that CXCL10 and IFN-γ are key players in OLP-associated inflammation, (Tao et al., 2008) and previous in vitro studies showed that multi-strain probiotics

are capable of suppressing LPS-induced chemokines, including CXCL10, through the blockade in the phosphorylation of the transcription factor STAT1. (Mariman et al., 2014) Clinically the disease presents with reticular hyperkeratotic changes of the oral mucosa, with more than 50% of the affected individuals also developing long-standing erosion and ulceration leading to reduced quality of life due to pain and dysfunction. (Osipoff et al., 2020) There remains no curative treatment and therefore management of OLP is aimed at reducing mucosal erosions/ulceration and the associated painful symptoms. Typically, this is achieved through the reduction of local T-cell inflammation and related inflammatory cytokines by anti-inflammatory medications, the most commonly used being glucocorticosteroids. Those not responding to glucocorticosteroids may benefit from immunosuppressant medications such as topical tacrolimus or systemic mycophenolate mofetil or azathioprine. (Carrozzo et al., 2019).

Systematic reviews and meta-analyses have suggested that there remains little robust evidence supporting the use of any of the above medications in the treatment of OLP. (Lodi et al., 2020; Yang et al., 2016) Toxicity profiles associated with long-term use of the above medications can also be significant: ranging from transient localised mucosal burning to gastrointestinal toxicity, recurrent fungal infections, adrenal or bone marrow suppression and increased risk of cancer.(Lear et al., 1995; Wee et al., 2012) As affected patients have also expressed concerns regarding the self-managed use and the adverse effects of currently available medications. (Ni Riordain et al., 2011) There is an unmet need to investigate alternative therapeutic strategies that may contribute to the long-term management of oral lichen planus, ideally with a safer profile.

There have been a number of recent papers that have demonstrated dysbiosis in the oral microbiome in OLP. (Wang et al., 2020) The results produced to date suggest that an alteration in the oral bacterial composition may be relevant to the development and progression of OLP, but the precise role played by the microbiome is still unclear. It is possible that targeting dysbiosis may have an impact on disease activity and could be a future therapeutic option in OLP.

Here, we present a preliminary clinical study aimed at investigating the potential benefit of probiotics as a novel strategy in the treatment of oral lichen planus. Probiotics are food products defined as a live microorganism that confers a health benefit to the host. They have been used in a range of medical conditions especially of the gastrointestinal tract including antibiotic-associated diarrhoea, irritable bowel syndrome, ulcerative colitis and pouchitis, with the aim of improving intestinal microbial balance, enhancing gut barrier function and reducing local and systemic inflammation. (Wilkins & Sequoia, 2017) In vitro and animal studies have suggested that probiotics can induce a reduction in pro-inflammatory cytokines. (Plaza-Diaz et al., 2014; Plaza-Diaz et al., 2017) Clinical studies have indicated possible clinical benefits in individuals with inflammatory gastrointestinal disease, although robust and conclusive evidence are still lacking. (Iheozor-Ejiofor et al., 2020; Kaur et al., 2020) A few small and uncontrolled studies have preliminarily assessed the potential beneficial effects of probiotics upon inflammatory diseases of the oral mucosa, although evidence remains weak, it has been suggested that the use of probiotics in individuals with oral ulceration of Behcet's disease, recurrent aphthous stomatitis and chemotherapy-related oral mucositis may lead to clinical benefits including reduced number of ulcers, subjective reduction in oral discomfort and reduced severity of oral mucositis. (Jiang et al., 2019; Rapoport & Levine, 1965; Tasli et al., 2006) To the best of our knowledge, there are two published studies investigating the use of probiotics as a treatment for OLP. (Keller & Kragelund, 2018; Li et al., 2020) The study by Keller and Kragelund in 2018, which looked at recurrent oral candidiasis in OLP, reported that the administration of the probiotic stain *Lactobacilli reuteri* to symptomatic patients did not reduce recurrent oral candidiasis or Candida count/carriage compared with placebo. (Keller & Kragelund, 2018) They did however state that across the entire study, the placebo group reported a higher visual analogue scale (VAS) pain score. In a more recent study by Li et al in 2020, it was shown that topical application of the probiotic *Streptococcus salivarius* K12 produced no adverse reaction in symptomatic OLP patients, but produced no significant clinical benefit (Li et al., 2020). The potential of probiotics as a treatment for OLP is still unclear and in need of further investigation. Therefore, before embarking on a large multicentre study, we propose that robustly designed preliminary work is needed in order to (a) demonstrate that some beneficial effect can be reasonably expected, (b) explore the potential mechanisms of action of the study intervention and (c) assess whether such a study would be feasible and acceptable to patients.

CABRIO (The clinical and biological effects of the use of probiotic in patients with oral lichen planus) was designed as a double-blind, randomised placebo-controlled proof of concept pilot trial of the probiotic VSL#3 in individuals with symptomatic oral lichen planus. The aims of the study included (a) gathering preliminary information on clinical benefits including perceived pain [primary outcome], disease-specific quality of life (QoL) and disease activity [secondary outcomes], (b) assessing safety and tolerability of the study intervention and participants' compliance to protocol and attrition [secondary outcomes] and (c) measuring the changes in pro-inflammatory cytokine levels and oral microbial composition as a measure of the underlying mechanisms of action of probiotics [mechanistic outcome].

The present report follows the CONSORT 2010 guideline extension to randomised pilot and feasibility trials. (Eldrige et al., 2016).

2 | METHODS

2.1 | Trial Design

CABRIO is a proof of concept parallel group randomised placebo-controlled (1:1) pilot trial of probiotics in the treatment of oral lichen planus, with embedded feasibility and mechanistic outcomes.

The study received favourable ethical opinion by NHS Health Research Authority and London–Queen Square NHS Research Ethics Committee (protocol 16/0622, IRAS ID 22017). This study was registered on ClinicalTrials.gov (NCT03052179) and NIHR Portfolio (CPMS ID: 34016). CABRIO is an investigator-led study sponsored by University College London (17/LO/0475) with funding support from the Industry (award number 174235 from PT. Ferring Co. Ltd). Additional infrastructure and service support funding were provided by the NIHR UCLH Biomedical Research Centre and NIHR Clinical Research Network. Funders had no role in study design or data analysis. With the exception of the additional measurement of interferon-gamma levels in the saliva, no amendments were made to the protocol after the study commenced.

2.2 | Setting, data and sample collection

Potential participants were identified through the oral medicine outpatient clinics at the Eastman Dental Hospital, University College London Hospital Foundation Trust between August 2017 and July 2018. Medical notes of attending patients were reviewed against the entry criteria, to identify potentially eligible individuals who were subsequently approached by the attending clinician and provided with verbal and written information on the study. Those who expressed interest in participating were invited to consent and attend a screening study visit for eligibility checking. Those meeting all the inclusion criteria and none of the exclusion criteria were offered recruitment into the study.

Study measurements and participant demographics were collected using a predefined case report form. Saliva and blood samples were collected at three time points including study visit 1 (baseline), study visit 3 (after 30 +/- 5 days) and study visit 4 (after 60 +/- 5 days). Saliva samples were collected in sterile tubes containing saliva preservative buffer (50 mM Tris, pH 8.0, 50 mM EDTA, 50 mM sucrose, 100 mM NaCl, 1% SDS) by the method of Quinque et al (Quinque et al., 2006) and stored at −80°C until analysed. Briefly, volunteers were asked to sit in a dental chair in an upright position for two–three minutes, before being asked to spit in to a sterile tube containing 2 ml saliva preservative buffer for up to 5 min or until the saliva reached 5 ml. Peripheral venous blood samples were collected into K2E (EDTA) BD vacutainer® (Becton Dickinson), centrifuged at 300 g for 20 mins at 4°C and the related serum was collected and stored at −80°C until analysed.

2.3 | Participant inclusion criteria

Inclusion criteria were: (a) biopsy-proven diagnosis of OLP as per WHO 1978 histological criteria (Rad et al., 2009) with no evidence of oral epithelial dysplasia or malignancy, (b) presence of painful intraoral symptoms associated to OLP at the time of recruitment/start of the intervention, with minimum severity of pain being ≥3 on a 0–10 numeric pain rating scale, (c) age >18 years and willing to participate in the study, (4) receiving no therapy or receiving best standard topical therapy (defined as self-managed use of topical analgesics, corticosteroids or immunosuppressants) at the time of recruitment.

2.4 | Participant exclusion criteria

Exclusion criteria were: (a) The use of systemic antibiotics, retinoid, corticosteroid or immunosuppressant agents within four weeks prior to enrolment in the study, (b) pregnancy or receiving IVF treatment, (c) known history of systemic disorders affecting the immune system, (d) active cancer or cancer in remission undergoing maintenance with chemotherapy or immunomodulatory agents, (e) evidence of oral epithelial dysplasia or oral malignancy on biopsy.

2.5 | Intervention and study protocol

The investigational study products included active multi-strain probiotic and placebo, which were packaged by the manufacturer in identical single-use sachets, all of the same size and colour. The active study product was a commercially available multi-strain probiotic brand named VSL#3 (VSL#3-ACTIVE Batch 703093 Exp. date 04/2019, provided by Actial Farmaceutica srl, Italy) containing >450 billion live bacteria per sachets (4.4 g). The strains contained within VSL#3 were listed as *Lactobacillus acidophilus* (BA05), *Lactobacillus delbrueckii* subsp. *Bulgaricus* (BD08) (reclassified as *lactobacillus helveticus*), *Lactobacillus paracasei* (BP07), *Lactobacillus plantarum* (BP06), *Bifidobacterium longum* (BL03), *Bifidobacterium infantis* (BI04) (BL03 and BI04 reclassified as *B. animalis* subsp.*lactis*), *Bifidobacterium breve* (BB02) and *Streptococcus thermophilus* (BT01). (Mora et al., 2019) The active products also contained maltose, corn starch and anti-cracking agent silicon dioxide while the placebo product consisted of maltose, corn starch and anti-cracking agent silicon dioxide but no probiotic. The presence of the same flavouring agents in both active and placebo products ensured no difference in flavour.

Participants in both the placebo and the active arm were instructed to take the study product at home, dissolve the contents of two sachets into cold water or fruit juice in the morning with breakfast and two in the evening with dinner and swallow it (four sachets per day in total), every day for 30 days. Participants were provided with a home diary to be completed twice a day with information regarding the time of the day when the study products were used and were also asked to return the study products that had not been used at the subsequent study visit.

Participants were allowed to use self-managed standard of care topical therapy during the study where needed, including topical analgesics, corticosteroids or immunosuppressants. Participants, who after commencing the trial, were prescribed systemic antibiotics because of an infection at any body site and those who were prescribed systemic corticosteroids or immunosuppressants because of unsatisfactory response to topical therapy were excluded from analysis. All adverse events and serious adverse events (expected

and unexpected) were collected and reported as per good clinical practice, with the exception of those events that were considered unrelated to the intervention.

2.6 | Primary outcome and primary outcome measure

The primary outcome was the change in intra-oral painful symptoms after 30 days of VSL#3 probiotic use with respect to baseline, as measured by pain numeric rating scale (pNRS). The pNRS was designed as a horizontal line with the numbers on a scale from 0, which represents no pain, to 10, representing the worst possible pain. This scale has been previously validated for measurement of the intensity of painful symptoms in OLP. (Escudier et al., 2007) For each study visit, participants were asked to mark the number on the pNRS according to their subjective experience over the previous two weeks including the day of the study visit.

2.7 | Secondary outcomes and secondary outcome measures

The secondary outcomes included change in intra-oral painful symptoms at 15 days and the end of the study defined as the pNRS score at final review visit (day 60 +/- 5 days, 30 days after stopping the intervention) compared with baseline, and the change in intra-oral painful symptoms over time, as measured by assessing pNRS scores at baseline, 15-day, 30-day and 60-day review visits in each randomised group. Secondary outcomes also included the change from baseline in Oral Disease Severity Score (ODSS) (Escudier et al., 2007) and quality of life (QoL) as determined by the chronic oral mucosal disease questionnaire (Ni Riordain et al., 2016; Ni Riordain & McCreary, 2011) at 15, 30 and 60 days, as well as over time.

In addition, a number of feasibility outcomes were considered including safety and tolerability of the intervention, and attrition/compliance with protocol, which were assessed by recording adverse side effects, completion of participant home diary, the number of returned unused study products, non-attendance to study visits, related missing measurements and withdrawals.

With respect to the mechanistic outcomes, we assessed the change from baseline in salivary and serum levels of the pro-inflammatory chemokine CXCL10 and IFN-γ at day 30 and day 60. IFN-γ and CXCL10 have previously been shown to be upregulated in OLP mucosal tissue, saliva and serum and may have an important role in regulating the inflammatory pathogenesis of OLP. (Abdel-Haq et al., 2014; Akpinar Kara, 2017; Deng et al., 2020; Di Lernia, 2016; Marshall et al., 2017; Nibali et al., 2012; Sun et al., 2005) An analysis of the changes in oral microbial composition from baseline and day 60 was conducted using 16S rRNA sequencing (for full details of the methodology see Supplementary Materials and Methods).

2.8 | Post-hoc exploratory outcome

Changes in topical corticosteroid usage over the duration of the trial were assessed in the two study arms as a surrogate of potential beneficial effects upon painful symptoms and the perceived need to use corticosteroid treatment.

2.9 | Sample size

A pragmatic sample size of 30 participants was chosen for this proof-of-concept pilot study. Recruitment of thirty participants was considered realistically achievable within the timeframe of this trial and the capacity of the oral medicine clinics at UCLH EDH, as well as adequate for the objectives of this preliminary study. (Julious, 2005).

2.10 | Randomisation, allocation concealment, implementation and blinding

A centralised computer-generated randomisation list was used to allocate participants to the active probiotics or placebo arm. An independent third-party statistician provided the electronic randomisation list, which included the identifiers to the study subjects and the treatment allocation, to the drug manufacturer who labelled the probiotics and placebo packages accordingly. All other aspects of the packaging were identical to ensure blinding. The randomisation list was blocked using varying block sizes and 1:1 allocation to the treatment and control groups. The emergency unblinding code was held in a password-protected electronic format on a secure password-protected drive by a member of the research team (SRP) who was not involved in participant recruitment and management, and was not viewable by other investigators until the database was unlocked. Paper copies of the randomisation key in closed envelopes, one for each participant, were also held by the same individual (SRP) and stored in a locked cabinet within a locked room with swipe card access system, to be accessed only in case of emergency unblinding.

Participants were recruited and allocated a study identifier and corresponding package of study treatment by research nurses. All study investigators recruiting or caring for trial participants did not have access to the randomisation list and were therefore blinded to the interventions. Packaging and labelling of active probiotics and placebo, as well as their administration, were identical between groups therefore ensuring participant blinding.

2.11 | Statistical analysis

Participant baseline data including demographics and clinical characteristics was summarised by randomised group. For the primary outcome (change in pain after 30 days), we compared the average change in pNRS score at 30 days between randomised groups

making adjustment for baseline using a linear regression model (analysis of covariance) (Douglas, 1990; Gardner & Altman, 1986; Vickers & Altman, 2001).

With respect to the secondary outcomes, we used a similar model to compare the mean change in pNRS score at 15 and 60 days between randomised groups adjusting for baseline and used graphs to examine the changes in mean pNRS score over time in each randomised group (including scores at baseline, 15-day, 30-day and 60-day time points).

Regression models were also used to compare the average changes in OLP disease activity score ODSS and QoL score at 15, 30 and 60 days adjusted for baseline and graphically examined the changes in mean ODSS and QoL score over time in each randomised group.

The feasibility outcomes were reported narratively and describing the occurrences of adverse events (safety and tolerability) and the withdrawal rate (attrition) summarised by randomised group using counts and proportions. We also reported descriptively on the changes in the use of corticosteroid therapy during the trial and the compliance to protocol by summarising the information obtained from participant diaries.

We compared the changes in mean levels of serum and salivary cytokines in each randomised group at 30-day and 60-day adjusted for baseline. For metagenomics analysis comparing salivary microbiome in individuals at baseline and day 60 as well as between groups, we used non-parametric statistical tests on the Bray–Curtis dissimilarity distances (full details can be found in Supplementary Materials). All analyses described in the main manuscript were as per protocol. For transparency and completeness, the intention to treat (ITT) analysis can be found in Supplementary Figure S1.

Statistical analysis was conducted using STATA (https://www.stata.com/) and significance set at $p < 0.05$. No correction for multiple testing was performed.

3 | RESULTS

3.1 | Participant flow, demographics and baseline measurements

We pre-screened against study criteria the medical notes of 1,748 consecutive OLP individuals attending the oral medicine clinic of UCLH Eastman Dental Hospital from July 2017 to June 2018. Of them, 1,393 were excluded (reasons provided in the CONSORT flow diagram) (Figure 1), and 355 were considered potentially eligible and approached in the clinic with information about the trial. Of these, 267 declined participation (age range 59 ± 11.7, due in part to a lack of availability to attend the required four study visits over a 60 day period. Thirty- five expressed an interest in the study and were consented and invited to attend a first screening visit for final eligibility checking. Of the 35 individuals who attended the study visit for eligibility assessment, 30 met all the inclusion and none of the exclusion criteria and were randomised into the placebo (n = 15) or probiotic (n = 15) arm. The first and last participants were randomised in August 2017 and July 2018 respectively. One participant in the placebo group and two in the VSL#3 group were withdrawn from the study intervention (on day 13, 11 and 14, respectively), as they required a course of antibiotic for dental (1 individual) and urinary tract infections (2 individuals). These three individuals were withdrawn from the treatment, but were required to attend the remaining study visits. Therefore, fourteen participants in the placebo group and thirteen participants in the probiotic group completed the trial intervention as per protocol and were included in the analysis. Demographics and baseline clinical measurements of all participants who completed the trial are summarised in Tables 1 and 2 respectively. The randomisation procedure resulted in no notable differences in demographics or clinical measurements between the placebo and VSL#3 groups at day 0 (baseline).

3.2 | Primary outcome (change in pNRS score at 30 days)

Both VSL#3 and placebo groups showed similar reductions in mean pNRS scores at day 30 compared with baseline, with no evidence of a statistically significant difference between randomised groups ($p = 0.749$, adjusted difference in mean pain score was 0.48 with 95% CI −1.46 to 1.12) (Figure 2A).

3.3 | Secondary outcomes

With respect to change in pNRS at 15 days with $p = 0.315$, adjusted difference in mean pain score was 0.69 with 95% CI −2.34 to 0.81 and 60 days (30 days after the end of the intervention) demonstrated no evidence of a difference in means between VSL#3 and placebo groups ($p = 0.412$ adjusted difference in mean pain score was 0.46 with 95% CI, −2.66 to 1.09) (Figure 2A). Furthermore, the mean changes in pNRS over time followed a similar trajectory in both groups, with a decline in mean pain score from baseline at days 15 and 30, followed by an elevation at day 60, with no statistically significant difference between the two groups at any of the time points (Figure 2A).

Analysis of the change in ODSS mean score showed no statistically significant differences between groups at 15 ($p = 0.723$ adjusted difference in ODSS was 0.75 with 95% CI −5.9 to 3.46), 30 ($p = .0.914$ adjusted difference in ODSS was 0.69 with 95% CI, −4.82 to 5.32) and 60 days ($p = 0.861$ adjusted difference in mean ODSS was 0.94 with 95% CI, −4.94 to 5.86) (Figure 2B). There was no difference in the ODSS mean scores between the VSL#3 and placebo groups over time (Figure 2B).

Analysis of the changes in QoL mean scores showed no statistically significant differences between groups at 15 ($p = 0.192$ adjusted difference in QoL was 0.94 with 95% CI −2.2 to 11.04), 30 ($p = 0.518$ adjusted difference in QoL was 1.12 with 95% CI, −5.78 to 10) and 60 days ($p = 0.96$ adjusted difference in QoL was 1.03

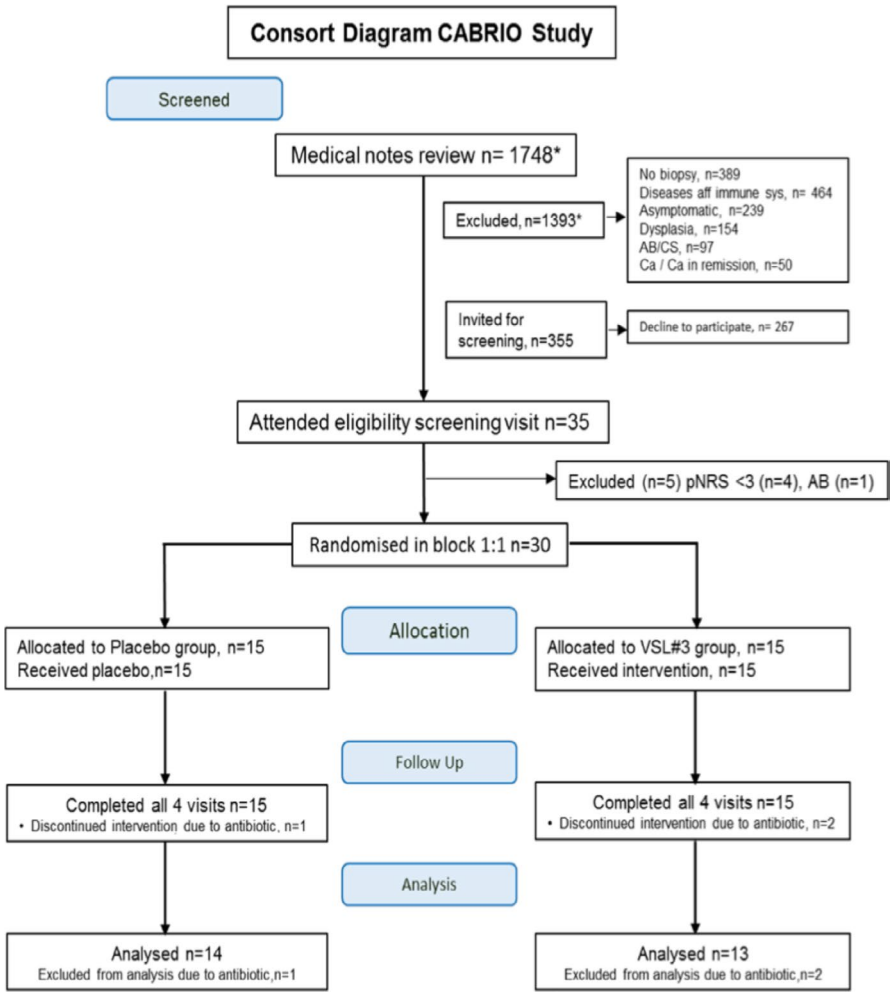

FIGURE 1 CONSORT (consolidated standards of reporting trials) flow diagram of CABRIO Study. AB, Antibiotic; CS, Corticosteroid

TABLE 1 Baseline demographic characteristics of CABRIO participants

Baseline Variable	Group Placebo		Group VSL#3	
	Recruited ($N = 15$)	Completed study ($N = 14$)	Recruited ($N = 15$)	Completed study ($N = 13$)
Age (Mean ± SD)	56.1 ± 11.8	55.1 ± 11.5	59.3 ± 8.3	59.5 ± 8.9
Gender (%)				
Female	12 (80)	11 (78.57)	12 (80)	10 (76.92)
Smoke (% Yes)	2 (13.33)	1 (7.14)	0 (0)	0 (0)
Alcohol in unit (%, weekly)				
0–5	13 (86.67)	12 (85.7)	11 (73.3)	10 (76.92)
6–10	1 (6.67)	1 (7.14)	4 (26.67)	3 (23.08)
11–15	1 (6.67)	1 (7.14)	0 (0)	0 (0)
Topical (% Yes)	11 (73.33)	10 (71.43)	12 (80)	11 (84.62)
Race (%)				
White	8 (56.67)	7 (50)	9 (60)	7 (53.85)
Asian	7 (46.67)	7 (50)	6 (40)	6 (46.15)

with 95% CI, −9.25 to 9.4) (Figure 2C). Analysis of the QoL mean score changes over time showed no statistically significant difference between groups at days 15 and 30 ($p = 0.192$ and $p = 0.518$, respectively).

3.4 | Feasibility Outcomes

There was very good compliance with the study protocol, the total attrition being 10% (3 out of 30 participants were withdrawn). All

TABLE 2 Baseline disease scores for the patients who completed the trial

Disease activity	Baseline Day 0 variables (Mean ± SD)		
	Placebo ($n = 14$)	VSL#3 ($n = 13$)	p value
pNRS	5.39 ± 2.11	5.27 ± 1.61	0.867
ODSS	26.6 ± 6.25	29.8 ± 10	0.342
QoL	52.43 ± 20.12	59.15 ± 15.6	0.642

Note: Students T-test.

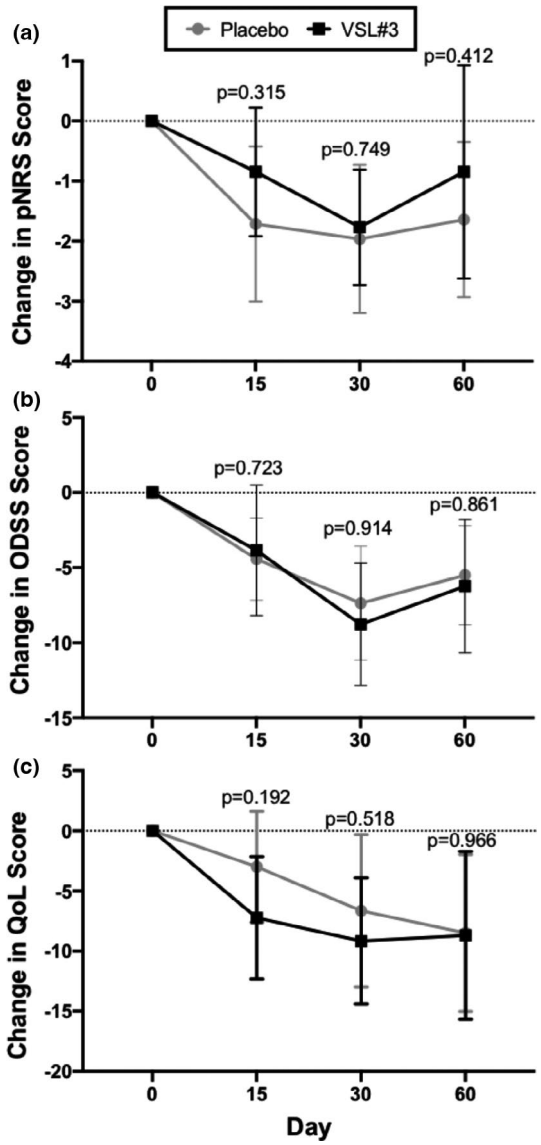

FIGURE 2 Alteration in clinical scores compared with baseline measurements over the trial period. Mean change in (a) pNRS, (b) ODSS and (c) QoL scores. p-values are from a comparison of randomised groups adjusting for the baseline score. Placebo group $n = 14$, VSL#3 group $n = 13$, Mean +95% CI

participants completed all study visits. Analysis of the participant home diary and the number of returned unused study products showed high compliance in both groups: 100%, 90–99% and 80–89% compliance were seen in four, nine and one participants of the placebo group and six, five and two participants of the active group respectively (Table 3).

With respect to the tolerability and safety of the intervention, the patients in the VSL#3 group showed the same proportion of adverse events as the placebo group (Table 3). Bloating, which is listed by the manufacture as an expected side effect of consuming VSL#3, was the most frequently reported adverse event in both the VSL#3 (n = 3) and placebo (n = 3) groups.

3.5 | Mechanistic outcomes

Analysis of changes in salivary CXCL10 levels for 30 and 60 days showed a weak (but not statistically significant) trend towards a reduction in the VSL#3 group after adjustment for baseline: difference in means (placebo- VSL#3) 0.15 with 95% CI −1.52 to 4, $p = 0.143$, and 0.14 with 95% CI −3.58 to 2.37 $p = 0.614$, respectively (Figure 3A). Changes in serum CXCL10 levels at 30 and 60 days also showed no statistically significant difference after adjustment for baseline: difference in means (placebo-VSL#3) 0.31 with 95% CI −0.86 to 5.67, $p = 0.894$, and 1.15 with 95% CI −2.5 to 5.85, $p = 0.77$, respectively (Figure 3B).

Analysis of the change in salivary IFN-γ revealed no significant alteration between the VSL#3 group compared with the placebo group at day 30 ($p = 0.082$, difference adjusted for baseline was 1.68 with 95% CI −0.66 to 0.2) (Figure 3C). The change between groups at day 60 was also not significant ($p = 0.587$, difference adjusted for baseline was 1.26 with 95% CI, −0.2 to 0.17). IFN-γ levels in the serum were too low to measure and therefore not analysed.

The analysis showed that the consumption of VSL#3 or placebo had no significant influence on saliva microbiome variation (Table 4). Although there were notable changes in the saliva microbiomes between day 0 and day 60, similar shifts were seen in both the placebo and the VSL#3 groups and there was no statistical difference between the two groups at either timepoint (Figure 4 and Table 4b). Overall, the 16S rRNA analysis showed that VSL#3 did not significantly impact on the composition of the salivary microbiome in OLP patients 30 days after consumption of the probiotic ceased.

3.6 | Topical corticosteroid usage

This post-hoc exploratory analysis showed that the total number of participants in the active and placebo group who used self-managed topical corticosteroids at any time point during the trial was 11 (out of 13) and 10 (out of 14) respectively (Table 1). The number of patients using topical corticosteroid, as well as the number of applications per day, dropped during the study in both the active and placebo groups (Figure 5). The difference between groups was not found to be statistically significant for the number of participants taking topical corticosteroids at day 30 or 60 ($p = 0.06$ and 0.071

TABLE 3 Associated/non-associated adverse events and compliance for CABRIO participants

GROUP	Patient Number	% Compliance (100% = 120 sachets)	Adverse Event	
			Associated	Non-associated
PLACEBO	1	100	-	Shoulder pain
	2	-	Withdrawn from study due to antibiotic use (UTI)	
	3	>100	-	-
	4	100	-	-
	5	100	-	-
	6	>100	Bloating	Cold
	7	100	-	Cold
	8	>100	-	Nausea
	9	100	Bloating/Nausea	-
	10	95	Bloating	Sinusitis
	11	81	-	Flatulence
	12	98	-	-
	13	100	-	-
	14	>100	-	Swollen gland
	15	93	-	Haemorrhoid operation
VSL#3	1	93	Bloating/vomit	Headache
	2	>100	-	CS injection
	3	81	-	Car accident
	4	>100	-	Stomach pain/cramp
	5	>100	-	-
	6	96	Bloating	Migraine
	7	86	-	-
	8	-	Withdrawn from study due to antibiotic use (Dental)	
	9	96	-	-
	10	>100	-	Migraine
	11	96	-	-
	12	>100	-	Stomach cramp, dental
	13	91	Bloating	-
	14	-	Withdrawn from study due to antibiotic use (UTI)	
	15	>100%	-	-

Note: >100% compliance achieved when participant consumed more than the required 120 sachets, which usually occurred when participants attended visit 3 between days 31 and 35.

Abbreviations: CS, corticosteroid; UTI, urinary tract infection.

respectively, Fisher's exact test, two-tailed) or in regard to the alteration in number of applications per day ($p = 0.347$ Student T-Test).

4 | DISCUSSION

CABRIO to our best knowledge was the first trial investigating the potential use of a multi-species probiotic supplement in the treatment of OLP. The protocol design also contained an embedded mechanistic study aimed at understanding the potential mode of action.

Our findings show that the highly concentrated probiotic VSL#3 was safe and well tolerated by patients with OLP. We observed no convincing evidence of a notable reduction in pain, disease activity or QoL at day 30 and 60 endpoints of the study in the active group with respect to placebo. However, the analysis of changes over time, which is particularly relevant for longitudinal studies of interventions for chronic diseases, (Senn et al., 2000) showed a reduction in mean QoL scores in the active group compared with placebo at days 15 ($p = 0.192$) and 30 ($p = 0.518$), although statistical significance was not reached. As an indirect surrogate signal indicating a potential effect of VSL#3 consumption, we observed a reduction in the number of participants in the active group taking topical corticosteroid at the 30-day endpoint, this was not significant when compared with the placebo group ($p = 0.06$).

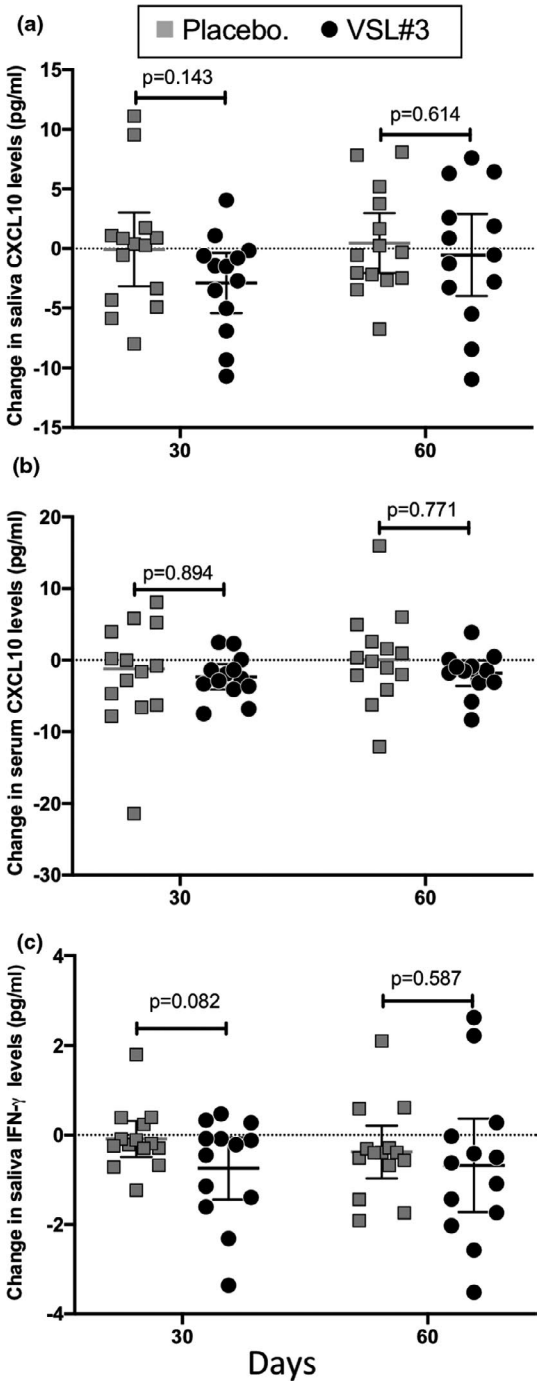

FIGURE 3 Alteration in pro-inflammatory cytokine levels compared with baseline measurements over the trial period. Change in (a) salivary CXCL10 levels, (b) serum CXCL10 levels, (c) salivary IFN-γ levels. Placebo group $n = 14$, VSL#3 group $n = 13$

There have been two previous published studies on the use of single species probiotics and OLP, which have reported similar results to our findings. Li et al, carried out a pilot randomised controlled study to evaluate *Streptococcus salivarius* K12 in the treatment of patients with symptomatic OLP. The participants were provided with either 0.1% triamcinolone acetonide dental paste or *S. salivarius* K12 lozenges 1 tablet twice daily (~1 billion CFU/unit of *S. salivarius*). They reported no significant difference in the VAS between the two groups after 4 weeks, in agreement with our findings. (Li et al., 2020) Keller et al also performed a double-blind randomised, placebo-controlled intervention study investigating the potential effects of probiotics in recurrent oral candidiasis in patients with OLP. (Keller and Kragelund, 2018) Lozenges containing *Lactobacillus reuteri* were consumed for 16 weeks and clinical parameters including VAS, severity score, *Candida* numbers, plaque and gingival index were reported. No difference in recurrent oral candidiasis, Candida count over time was seen with probiotic treatment. Gingival index decreased in the probiotic group and increased in the placebo group and over the entire study the placebo group produced a higher VAS pain score. The disparity in VAS results between the three studies could be due to a number of factors, such as mode of delivery, patient populations, presence of oral candidiasis, probiotic species used, dosage levels and duration of consumption. Further studies are needed to determine the potential of probiotics in the treatment of OLP and associated conditions.

With respect to the mechanistic outcomes, the analysis of changes in salivary levels of CXCL10 showed no significant reduction in the active group. The changes in salivary levels of IFN-γ between groups showed a reduction in the active group at 30 days ($p = 0.08$), which was not statistically significant. It is important to mention we did not measure salivary flow rate in the participants during the study. Salivary flow rate is known to be abnormal in OLP and this may have impacted on our findings associated with saliva cytokine levels and microbiome composition. (Mansourian et al., 2018) Future studies would benefit from recording the salivary flow rates of participants during the trial period.

There are a number of factors that may explain the lack of more robust results and statistical significance of our findings. CABRIO was designed as a proof-of-concept trial with a pragmatic sample size and as such it was not specifically powered to detect statistically significant meaningful changes related to clinical efficacy. There were also a number of measurements and information that was not included as part of the proof-of-concept study that may have influenced the findings, such as saliva flow rate, the presence of secondary oral candidiasis both of which are known features of OLP. It is possible that both saliva flow rate and candidiasis could impact on disease activity, cytokine levels and microbiome composition. It is also possible that probiotic consumption could alter the fungal populations within the saliva as well as saliva flow rate. Follow on studies would benefit from including these two additional parameters. The mode of administration of the product was not standardised for participants and different consumption regimes might have contributed to variability in results. Participants were requested to either pour the sachet content on their food, drink or to eat it directly. Although specific instructions were provided to each participant requesting, they do not add the product to carbonated soda, hot food or hot drink, no further instructions were provided. It is therefore possible that some variation in mode of consumption exists and this could impact on the findings. Future studies would benefit from a standardised mode of consumption for all participants.

TABLE 4 Comparison of saliva microbiome composition (A) between baseline and day 60 for VSL#3 and placebo groups, (B) between VSL#3 and placebo groups at baseline and day 60, using three non-parametric statistical tests. PERMANOVA, ANOSIM and MRPP tests on Bray–Curtis distances. All three tests showed that the consumption of placebo or VSL#3 had no significant influence on saliva microbiome variation on days 0 and 60 of the study

A. Variable	Non-parametric statistical test (p-value) Day 0 and Day 60 saliva microbiome composition		
	PERMANOVA	ANOSIM	MRPP
Placebo	0.862	0.920	0.864
VSL#3	0.946	0.984	0.994
B. Variable	**Non-parametric statistical test (p-value) saliva microbiome composition between VSL#3 and placebo groups**		
	PERMANOVA	ANOSIM	MRPP
Day 0 (Baseline)	0.952	0.847	0.941
Day 60	0.801	0.683	0.610

Significance reached at $p < 0.05$.

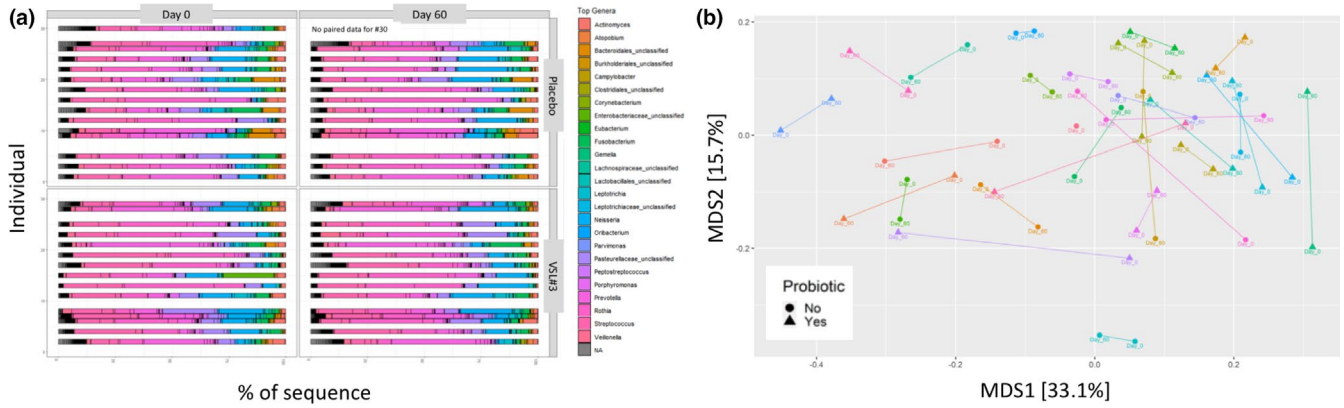

FIGURE 4 An analysis of salivary microbiome variation between samples taken on Day 0 and Day 60. (a) Difference in relative abundance of the top 25 genera between day 0 and day 60. (b) Multidimensional scaling/principal coordinates analysis (MDS/PCoA) was carried out separately on saliva samples taken from placebo-prescribed (•) and VSL#3-prescribed (Δ) individuals. Paired samples from the same individual are connected by solid lines. Placebo group $n = 14$, VSL#3 group $n = 13$

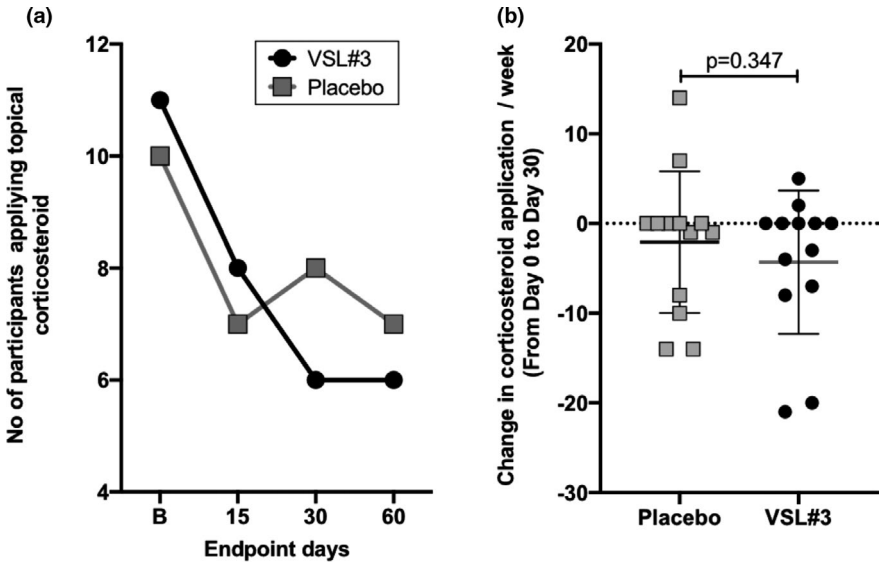

FIGURE 5 Change in corticosteroid usage. (a) Change in number of participants applying daily topical corticosteroid. (b) Reduction in number of topical corticosteroid applications per day per individual at day 30 of the clinical trial. Analysis by Fisher's exact test. Placebo group $n = 14$, VSL#3 group $n = 13$

The provision of study intervention was designed as potentially adjuvant, as participants were allowed to use their standard of care topical corticosteroid therapy during the trial as needed. This may have masked or reduced the magnitude of the intervention effects upon participants' symptoms and disease activity. Interestingly, we observed a trend, which did not attain statistical significance, towards a reduction in the number of participants in the active group self-administering topical corticosteroid during the trial ($p = 0.06$), which suggests that individuals taking the active probiotic may have perceived a reduced need to use their standard therapy as they progressed in the trial. It is possible that this was a consequence of some degree of reduction in disease activity and painful symptoms, albeit with a magnitude that was too small to be captured by changes in the pNRS or ODSS scores, but meaningful enough to patients so to determine a subjective reduction in the use of standard topical corticosteroid therapy. We did not record the volume of topical corticosteroid applied at each application and this could differ between subjects and have an influence on the findings. Larger and longer studies will be needed to verify these statements.

Interestingly, we did not observe notable changes in the salivary microbiome on day 60 as compared with baseline or between groups, therefore VSL#3 does not seem capable of changing the composition of the salivary microbiome when measured a month after consumption was stopped. The potential biological effects of VSL#3 on CXCL10 and IFN-γlevels, and clinically upon QoL scores and the perceived need of using topical corticosteroids may require continuous consumption of the product, as we observed no improvement in cytokine levels or clinical scores between days 30 and 60. The observed reduction in QoL scores and in saliva cytokine levels were recorded on days 15 and 30, which coincided with the consumption of VSL#3. Interestingly, the control group responded in a similar manner, which suggests either a placebo effect or a general response to the application of corticosteroids that all participants were permitted to self-administer during the study. The perceivable placebo effect in the participants will need to be considered when designing future clinical studies in OLP. The inclusion of an untreated control group in any future study would allow the true placebo effect in this population to be determined. A longer and larger study is needed to determine if continued VSL#3 consumption provides additional benefits to symptomatic OLP patients.

5 | CONCLUSIONS

The present proof-of-concept study does not provide sufficient evidence to support the notion that VSL#3 has any biological or clinical effects in individuals with painful lesions of OLP. It remains unclear whether the lack of more encouraging and robust results is due to a genuine lack of beneficial effects in this patient population or other factors unrelated to investigational product. The latter include the proof-of-concept nature of the trial, which was not designed or powered to demonstrate efficacy, and the use of VSL#3 as an adjuvant treatment along with standard of care topical corticosteroid therapy. The preliminary results of CABRIO suggest that further research in this field is warranted and provides useful information with respect to aspects of the design of a subsequent phase II study.

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CONFLICT OF INTEREST

None to declare.

AUTHOR CONTRIBUTIONS

Erni Marlina: Data curation; Formal analysis; Investigation; Project administration; Writing-original draft. **Richard N. Goodman:** Data curation; Formal analysis; Writing-original draft. **Valeria Mercadante:** Supervision; Writing-review & editing. **Martina Shephard:** Investigation; Supervision. **Roddy McMillan:** Supervision. **Tim Hodgson:** Supervision. **Rachel Lesson:** Investigation; Supervision. **Stephen Porter:** Investigation; Project administration; Supervision. **Julie A. Barber:** Conceptualization; Data curation; Formal analysis; Methodology; Project administration; Supervision; Writing-original draft. **Stefano Fedele:** Conceptualization; Data curation; Investigation; Methodology; Project administration; Supervision; Writing-original draft. **Andrew M. Smith:** Conceptualization; Funding acquisition; Investigation; Methodology; Project administration; Supervision; Writing-original draft.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/odi.14014.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

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