# POLARIS (Polybactum® to Assess Recurrent Bacterial Vaginosis): Open-label, Non-controlled, International Clinical Study with Extended 10-month Follow-up without Treatment

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## Abstract

Recurrent bacterial vaginosis (RBV) after antibiotic treatments has a relapse rate of 35% within 3 months and 60% within 12 months. Products containing polycarbophil (PLGG), that inhibits bacterial growth and mucoadhesive property, can impair biofilm formation. Here are shown the results of the POLARIS (Polybactum® to assess Recurrent Bacterial Vaginosis) study.

The first phase was an interventional, open-label, non-controlled, and multicentre trial enrolling 56 women in Italy and Romania. The second phase was an observational 10-month follow-up without treatment conducted only in Romania.

After 3 cycles with PLGG, only 8 BV recurrences out of 54 evaluable patients were identified (rate 14.81%) and for 26 out of 39 patients (66.67%) was evidenced positive effect on *Lactobacilli* in the vaginal secretions. In the follow-up 35 patients were observed after PLGG stopping treatment; 1 RBV (2.86%) at the 4$^{th}$ month and an additional 6 cases (17.14%) were evidenced at the end of the follow-up period. Therefore, no recurrence was evidenced in 12 subjects (34.28%) at 10$^{th}$ ± 2 months after the end of the PLGGtreatment.

The use of PLGG vaginal ovules in the treatment of BV reduces the rate of relapses and evidenced a positive effect on *Lactobacilli* in 66.7 % of cases, improving the microbiological parameters.

**Keywords:** bacterial vaginosis; recurrent vaginitis; biofilm; polycarbophil

## 1. Introduction

The vaginal microbiome, characterized by the dominance of the bacterial species *Lactobacilli*, can change its composition under the influence of a range of exogenous and endogenous factors, able to increase vaginal pH and produce unfavourable conditions for the survival of *Lactobacilli.* Then, the altered vaginal ecosystem allows the replacement of *Lactobacilli* by anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae*, *Ureaplasma urealyticum*, *Mycoplasma hominis*, and others [1]. The disruption of the normal vaginal flora produces dysbiosis, such as bacterial vaginosis (BV), which is the most common cause of abnormal vaginal discharge during childbearing age, representing around 30% of all cases [2, 3]. Moreover, BV has been linked to serious conditions,

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including pelvic inflammatory diseases, postoperative infections, acquisition and transmission of human immunodeficiency virus, preterm birth, and several adverse pregnancy outcomes [4]. Even if 50% of women have no symptoms, the most reported complaints are abnormal watery, grey vaginal discharge, and fishy odour. The diagnosis is founded on the presence of three out of four Amsel criteria (high vaginal pH, abnormal grey discharge, positive amine test, and greater than 20% clue cells on saline microscopy), with a sensitivity of 92% [5]. The aetiology and pathogenesis of BV are not completely understood, due to the several risk factors having a determining role, thus the available treatments are not yet always effective, resulting in a high recurrence rate [6]. Recurrent Bacterial Vaginosis (RBV) is defined as 2-3 episodes of BV per year [7]. RBV and abnormal vaginal flora can be associated with a history of BV, a regular sex partner, and female sex partners; the use of hormonal contraception had a negative association with recurrence. Recurrence rate reported reach 35% within 3 months [8], 50% within 6 months [9], and 60% within 12 months [11]. It was proven that the tendency to relapse is essentially linked to the ability of *Gardnerella vaginalis* (and to a lesser extent of *Atopobium vaginae*) to strongly adhere to the mucous membranes producing a long-lasting biofilm [10, 11].

Metronidazole (systemic or topical), tinidazole (systemic), and clindamycin (systemic or topical) are equally suggested as a first-line medical approach [12], but although standard antibiotic regimens can decrease the load of pathogens, it was demonstrated that bacteria biofilm can persist on the vaginal epithelium, thus setting the stage for recurrence after treatment. For this reason, it is suggested by several studies [11] that the employment of products able to create a barrier effect against the bacterial biofilm formation and permanence could be useful to prevent recurrences. These elements (i.e., lysozyme, thymol, bromelain, and polycarbophil) are widely used in the daily practice of thousands of gynaecologists in Europe with the caveat to administer them no later than 24 hours after the antibiotic treatment. We have performed a systematic review in the literature on products able to create a barrier effect against the bacterial biofilm formation, evidencing that they can improve the results of BV treatment. Unfortunately, there are little data supporting the decrease of recurrence rate with their administration after antibiotic therapy.

This study reports the results of the POLARIS (Polybactum® to assess Recurrent Bacterial Vaginosis**)** study that was planned to evaluate the ability of a medical device containing polycarbophil, lauryl glucoside, and glycerides (PLGG) to reduce BV recurrence rate during follow up period after one course of antibiotic therapy. The tested product (Polybactum®, Effik Italia S.p.a., Cinisello Balsamo, Milan, Italy) is already on the market as Class IIa medical device in several European countries. The rationale for its use relates to its specific bacteriostatic action, which inhibits bacterial growth, and to its mucoadhesive property, which can impair the formation of the biofilm produced by *Gardnerella vaginalis* and other bacteria. Furthermore, PLGG has an acidifying action on the vaginal pH which improve the microbiological parameters and maintains a hostile environment for the recolonization of the vagina by the polymicrobial flora involved in BV [13]. PLGG has shown to have no effect in terms of systemic tolerance, due to its inability to cross the epithelium, and a good local tolerance even after prolonged exposure: it does not cause any irritation to the vaginal wall, has no toxic effect on the epithelial cells, and does not trigger any sensitivity reaction [14,15].

## 2. Materials and Methods

*Study design*

The POLARIS study was planned with an open-label, non-controlled design in Italy and Romania. In the first phase, 3 cycles of treatment with PLGG were administered, followed by 1 month of follow-up without treatment. In the second phase the follow-up without treatment continued for additional nine months in the three Romanian centres, only (www.clinicaltrials.gov as NCT 02863536). Therefore, each patient could have had a

follow-up period of 10 months without treatment: 30 days from the end of the 3rd cycle of PLGG to day 78±6 days and 9±2 months from that date to the final visit. In the follow-up, only subjects that have completed the first phase and voluntarily give their consent also to the observational part of the study were included.

The study received formal approval by the National Agency for Medicines and Medical Devices of Romania (*Agentia Nationala a Medicamentului si a Dispozitivelor Medicale*) and was notified to the Italian Ministry of Health. The local Ethics Committees (ECs) of the investigational sites approved the study in Romania (on July 29th, 2016, the *EC Clinica Medicală Dr. Crișan*, on October 24th, 2016, the *EC Clinica Medicală Dr. Biriș*, and on August 22nd, 2016, the *EC Clinica Medicală Dr. Sîrbu*, all located in Timisoara) and in Italy (on December 14th, 2016, the *EC Area 1* in Milan and on February 15th, 2017, the *EC Lazio 1* in Rome). The study was amended (on March 14th, 2018) to allow the follow-up period without treatment and to perform an interim analysis 6 months after the start of enrolment, whose results have been recently published [16].

*Participants*

Fifty-six women older than 18 years and affected by RBV were included in the interventional first phase of the study. Diagnosis of BV was based on Amsel criteria [17] performed in the 6-9 days before baseline. Antibiotic therapy was represented by vaginal metronidazole (5 g of 0.75% gel once daily for 5 days or 500 mg ovules once daily for 7 days). The inclusion criteria were RBV (at least 2 episodes of BV in the last 12 months, including the episode treated before baseline), above 18 years, the signed informed consent form (ICF) and the status of non-lactating or lactating not amenorrhoeic women. The exclusion criteria were: known allergy to metronidazole or the tested PLGG ingredients; pregnancy; candidiasis or mixed vaginitis; HIV or other immunodeficiencies; sex workers; ongoing menstrual bleeding or premenopause / menopause; patients concomitantly included or having participated in the previous month in any other interventional clinical study; unwillingness to provide the informed consent; time between the last day of last menses and baseline visit >16 days or ≤5 days. This criterion was necessary to avoid bias in case of a menstrual bleeding occurring during the first PLGG cycle and the need to interrupt the tested product administration.

Thirty-five patients, who completed the interventional phase of the study without any recurrence, gave their consent to be included in the observational follow-up, thus having a total period of 10 months without any treatment.

Two participating sites were Italian (coordinator site *Vittore Buzzi Hospital* in Milan and *AIED Centre* in Rome) and three sites were Romanian private clinics specialised in gynaecology (*Clinica Medicală Dr. Crișan*, *Clinica Medicală Biriș*, and *Clinica Medicală Dr. Sîrbu*). Following the indications recently reported in the clinical research literature [18] the trial was performed by a partnership between an academic research organization (*Department of Clinical Trials of the University of Medicine and Pharmacy Victor Babeș* in Timisoara, Romania) and an independent international Contract Research Organization (Opera CRO, a Tigermed Group company, Timișoara, România). In this model, the University Department was the direct contact with the Investigator Coordinator of the study at Vittore Buzzi Hospital in Milan and was involved in the selection and training of Investigators and Co-Investigators of the sites. The University Department also assured the correct application of ethics requirements and shared the scientific information throughout the study period. On the other hand, the CRO provided the infrastructure for efficient trial execution, site monitoring, data management, and statistical analysis. The model assigning the scientific activity to the Investigators linked to the University Department, whereas the management responsibility is assigned to the CRO, is also able to clear the charge of the Sponsor. In fact, the role of the Sponsor should be only to offer the tested medical devices and a partial grant as support to the trial, without any potential misunderstanding related to influence or potential persuasion in the study design, data collection, interpretation, or sharing for publication.

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For administrative reasons, the centres in Italy could only participate in the first interventional phase of the study and so the patients enrolled in two Italian sites were not included in the follow-up period.

The trial was carried out from September 8th, 2016 (First Patient First Visit) to October 18th, 2018 (Last Patient Last Visit).

*Interventions*

Before any procedure related to the study, each patient was informed about the purpose of the trial, the risks, and the benefits, and was requested to give her consent signing an ICF. An additional detailed ICF was signed by the patients at the beginning of the follow-up period. The administration of PLGG vaginal ovule started within 12 and 24 hours after the end of metronidazole vaginal treatment and continued for 3 menstrual periods (minimum 72 and maximum 84 days). The duration of each cycle of treatment was 1 week, with the tested medical device administered as follows: one ovule inserted in the vagina on day 1, one ovule on day 4, and the last ovule on day 7. At the baseline visit, the Investigator delivered to each patient 9 ovules for the whole study duration (3 ovules for 3 cycles). The Investigator also advised the patient to lay in a supine position for a couple of minutes after the ovule had been inserted. The baseline visit and the first cycle of PLGG vaginal ovule fell within the 6th and the 16th day after the menstrual bleeding and after the end of metronidazole treatment. In the second and the third cycle, PLGG vaginal ovule was administered immediately after the end of the previous menstrual bleeding. In any case, the Investigator could decide to stop administering the medical device for safety purposes or to prescribe other therapies if considered necessary for the patient's health. The following treatments were not allowed: other products or medication to treat BV; etonogestrel / ethinyl oestradiol vaginal ring (e.g., Nuvaring®, Merck & Co., Inc, Kenilworth, NJ, USA) or intrauterine devices; vaginal tampons; oral or vaginal antibiotic treatments or other vaginal therapies (like spermicide, douching); vaginal or oral probiotics (e.g., vaginal *Lactobacilli*).

*Primary and secondary outcomes*

The study included a baseline visit (day 0), a visit after 30 days after stopping PLGG treatment (day 78±6), and a visit at the end of the follow-up period (month 10±2). Three phone contacts with the patient were planned during the interventional phase of the study: at day 28 ±1 day after the last day of last menses, 28 ±1 days after the 1st phone contact, and 28 ±1 days after the 2nd phone contact. Additional two phone contacts were requested in the 4th and 7th months in the follow-up period. During any phone contact, the Investigator checked if the patient had BV symptoms and, in this case, scheduled an unplanned visit to confirm the diagnosis of BV based on Amsel criteria.

The primary outcome was the recurrence of BV identified by Amsel criteria [17], determined in planned or unscheduled visits. A positive diagnosis of BV required 3 of the following 4 criteria: vaginal pH greater than pH 4.5; the proportion of clue cells ≥ 20% of total epithelial cells in the vaginal fluid; the presence of white and thin vaginal discharge and fishy odour at whiff test. The secondary outcomes were vaginal *Lactobacilli* microbiota assessed by optical microscopy during the visits to evaluate the rate of normal vaginal microflora [19] after treatment; signs and symptoms of BV (vaginal discharge, erythema, burning, dyspareunia). Vaginal discharge over the last 24 hours was measured using this analogic 3-point scale: 0 = not present or physiological in quantity, colour, and type; 1 = mild abnormal (abnormal quantity with normal colour and type); 2 = abnormal quantity, colour, and type. Erythema was evaluated using the analogic 5-point scale: 0 = no symptoms; 1= slight; 2 = moderate; 3 = marked; 4 = very marked. Burning intensity over the last 24 hours was assessed using an analogic 5-point scale: 0 = not present; 1 = mild; 2 = moderate; 3 = severe; 4 = unbearable. Dyspareunia was evaluated by a dichotomic scale: 0 = absent; 1 = present. All secondary outcomes were measured considering value changes

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from baseline. The secondary outcome also included the patient's assessment of the global efficacy of treatment at the last visit using a 4-point scale: 1 = very good improvement; 2 = good improvement; 3 = moderate improvement and 4 = negligible improvement.

Safety was evaluated by collecting and evaluating the adverse events that occurred during the study period and by a global assessment of safety performed by Investigators using an analogic 4-point scale: 1 = excellent, 2 = good, 3 = fair and 4 = poor.

*Sample size determination and statistical methods*

It was considered that, according to published data, the mean recurrence rate of BV after a first episode was from 30 to 50% within 3 months after the appropriate antibiotic therapy [10]. Patients were not allowed to use any oral or vaginal antibiotic therapy after metronidazole treatment for the 3-cycles PLGG (that means about 3 months). So, it should have been realistic to have a mean recurrence rate of 40% also in this study. In addition, data previously collected on recurrence rate post-treatment with PLGG vaginal ovule (Effik Italia SpA, unpublished data), evidenced a 2% correlation between paired observation and after applying continuity correction, thus the study would have been required a sample size of 44 pairs to achieve a power of 80% and a one-sided significance of 5% for detecting a difference of 0.25 between marginal proportions. We evaluated a potential drop-out rate of 20%, and so we decided to enrol 55 patients (one group Chi-square test than a proportion equals user specified value non-inferiority). At 95% confidence interval, the level of significance of <0.5 was considered statistically significant.

The values between visits for the primary objective were analysed by a t-test or Chi-squared test for quantitative variables; in case of binary variables or qualitative variables respectively a McNemar test or a symmetry test were applied. The time-to-event for BV recurrences was analysed by Kaplan Meier curves. All tests were two-sided, and the nominal level of $\alpha$ was 5%; p-values were not adjusted for multiplicity in the case of the primary outcome. For secondary objectives, statistical tests include but are not limited to, Chi-square and McNemar's tests for categorical data, and a (non) linear mixed effects model analysis. Statistical analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

## 3. Results

### *3.1. Patient disposition*

Fifty-six patients were enrolled and started the interventional phase of the study. Two patients were excluded from analysis because they did not complete the PLGG vaginal ovule administration cycles (dropout not related to safety issues), and 54 patients were considered evaluable. Due to administrative reasons, the patients belonging to the Italian centres were not included in the follow-up and therefore only 35 patients were considered evaluable in this second phase of the study. See the flow chart in Figure 1.

**Table 1.** Demography of all patients (Treated population).

| Characteristics | # |
|---|---|
| Sex | 56 females |
| Age (years) | Mean±SD: 30.8±6.57 |
| | Min/Max: 18/ 44 |
| BV in the last 12 months | 2 episodes: 37 (66.07%) |
| | 3 episodes: 16 (28.57) |
| | 4 episodes: 3 (5.36%) |

### *3.2. Demography and baseline data*

No relevant findings were recorded in the medical history and during the physical examination. The baseline demographic data of patients and the number of episodes of

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BV in the last 12 months before enrolment are reported in Table 1. No relevant concomitant medication was recorded during the screening evaluation or during the study period.

*3.3. Primary efficacy outcome*

According to the analysis of the primary objective, 8 recurrences out of 54 evaluable patients were identified at the visit of day 78±6. Therefore, after the administration of metronidazole and 3 cycles with PLGG vaginal ovules, the recurrence rate of BV was 14.81% (8 of 54 subjects).

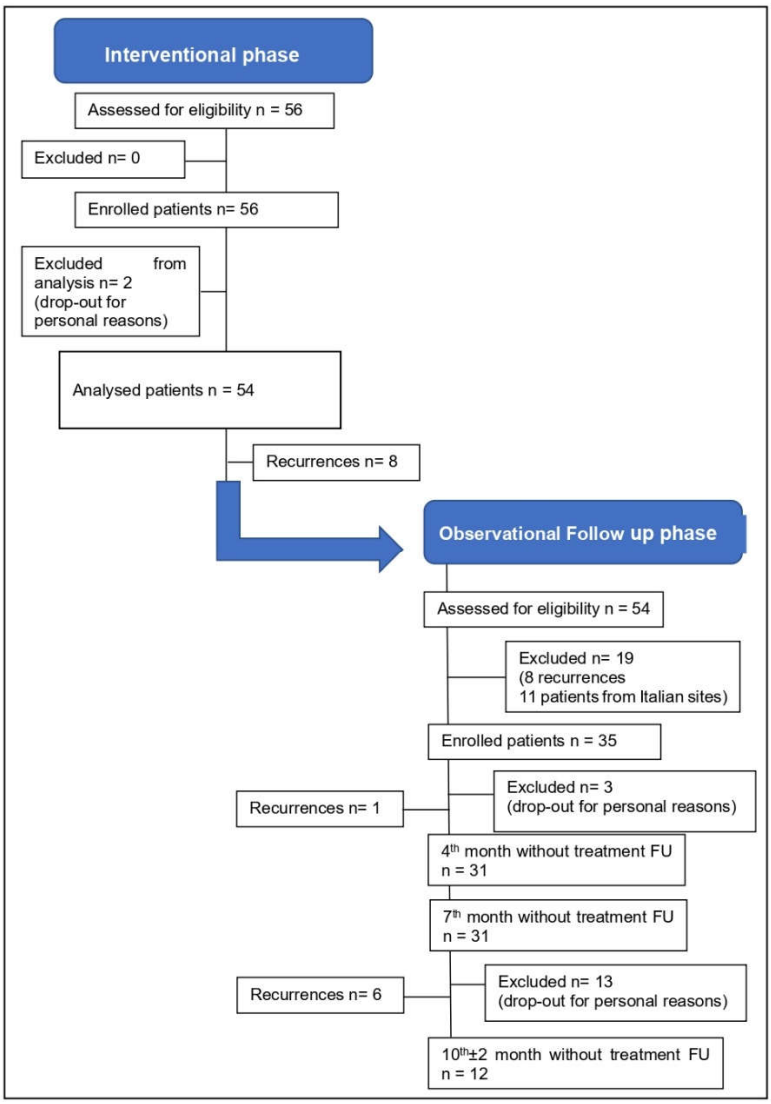


**Figure 1.** Flow diagram of POLARIS study.

These results show a significant statistical decrease with respect to the 40% reported in the literature and confirm the results previously evidenced in the interim analysis [16]. Then, the 3 months 14.81% BV recurrence rate must be related to the treatment period with PLGG vaginal ovule for 2 months plus 1 month without any treatment.

In the 35 patients who have completed the interventional phase of the study without any recurrence, and continued the follow-up period without treatment, the number of RBV cases was 1 (2.86%) in the 4$^{th}$ month. This rate was maintained for 6 months when additional 6 cases (17.14%) were evidenced at the final visit (10$^{th}$ ±2 months). Therefore, considering the 17 patients that totally drop out (48.57%), no recurrence rate was evidenced in 12 subjects (34.28%) after 10$^{th}$ ±2 months after PLGG stopped treatment. These

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data confirm and demonstrate the effectiveness of PLGG vaginal ovule previously administrated in reducing the rate of relapses even in the long term.

The Kaplan-Meier curve (Figure 2) highlights graphically the reduction during the time of the % of patients without RBV, and in Figure 3 the comparison in the recurrence rate per BV for each visit is shown. Treatment compliance rate (defined as the total number of administered ovules from the total number of given ovules in the interventional study) was 97.82% (493 out of 504 ovules) for all the patients enrolled, and 100% (486 out of 486 ovules) considering only the subjects who completed the study.

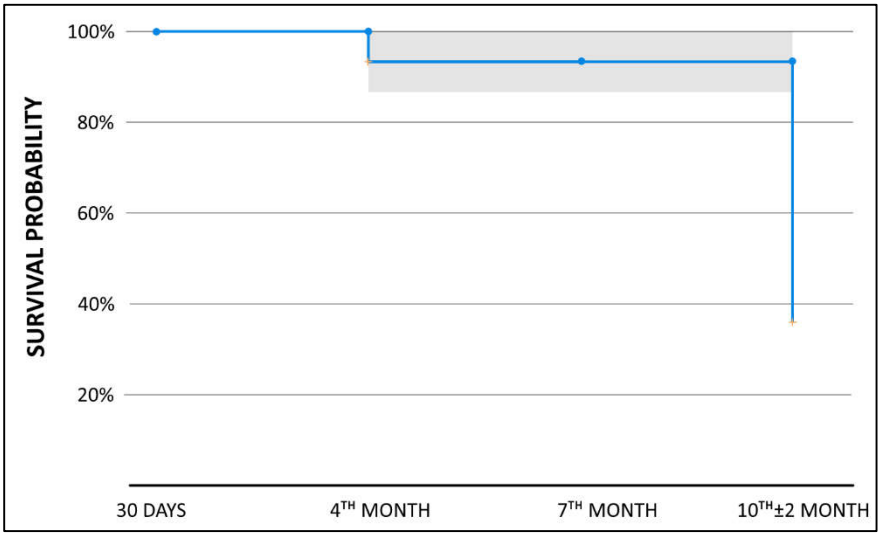


**Figure 2.** Kaplan-Meir curve after 30 days, 4, 7, and 10±2 months from PLGG stopping treatment.

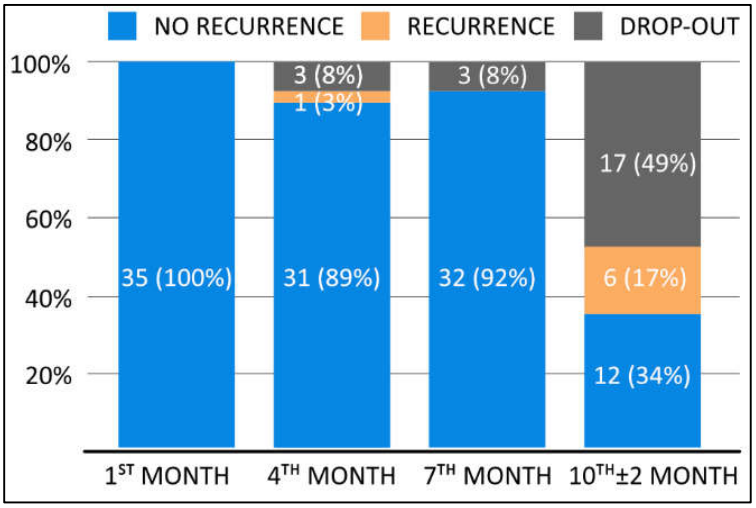


**Figure 3.** Bacterial vaginosis recurrence rate changes between visits after PLGG stopping treatment.

*3.4. Secondary efficacy outcomes*

The evaluation by phase-contrast microscopy of *Lactobacilli* microbiota [20] present in the vaginal secretions was measured at baseline visit and after 3 cycles of PLGG treatment, from the lowest to the highest concentrations, according to the following 5-point scale: absent, 1+, 2+, 3+, 4+. Data were collected only for Romanian sites for both interventional study and observational PMFU study (only for 2 out of 3 Romanian sites for the latter). Data collected for 39 subjects out of a total of 41 evidence for 26 patients (66.67%) that the treatment had a beneficial or neutral effect on the *Lactobacilli* concentration levels and only for 13 patients (33.33%) the concentration levels worsened (Chi-squared test, 5%

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significance level: p-value=0.054). Assessing the status-quo of the *Lactobacilli* concentration levels between the baseline and final visit of the interventional study, statistically, significant differences for the *Lactobacilli* concentration levels, between visits, were shown (Stuart-Maxwell asymptotic marginal homogeneity test, 5% significance level: p-value=0.011). The effect on *Lactobacilli* concentration level change between visits is graphically presented in Figure 4.

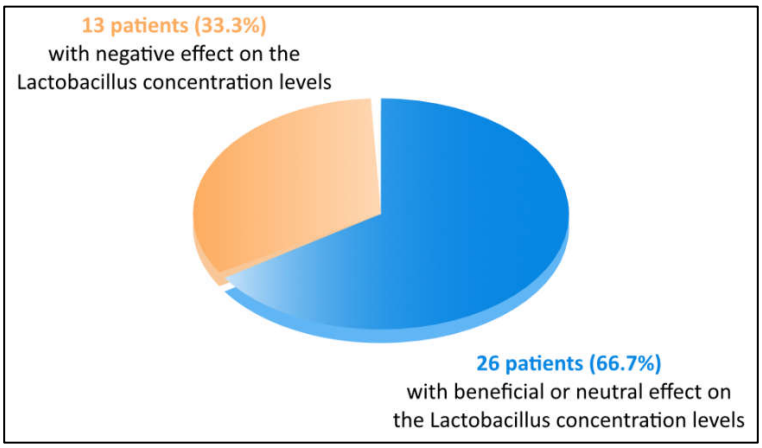


**Figure 4.** *Lactobacilli* concentration change in the interventional phase of the study, from baseline to day 78±6 visit.

Data were also collected for 17 subjects that completed the follow-up and attended the final visit on month 10$^{th}$ ±2 (Figure 5). There were no statistically significant differences in *Lactobacilli* concentration level change between visits, at a 5% significance level, according to the Maxwell-Stuart asymptotic homogeneity test (p= 0.306).

Results related to symptoms associated with BV (vaginal discharge, burning, erythema, and dyspareunia) in the first phase of the study are reported in Figure 6. The patient's global assessment of efficacy at day 78±6 was rated as "very good" by 74.07%, "good" by 12.96%, "moderate" by 7.41%, and "negligible" by 5.56% of patients.

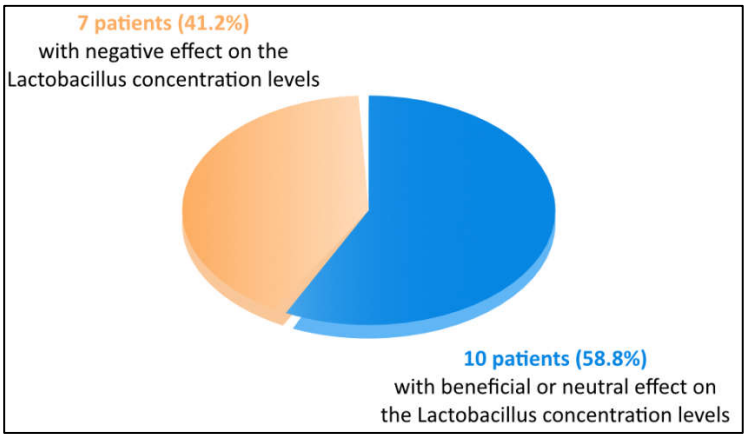


**Figure 5.** *Lactobacilli* concentration change after PLGG stopping treatment, from day 78±6 to month 10±2 visit.

### *3.5. Safety outcomes*

The Investigators' global assessment of safety was rated as "excellent" by 83.64%, "good" by 10.91%, and "fair" by 5.45% at day 78±6. Only 2 adverse events were reported in the first interventional phase of the study and no adverse event was evidenced during the follow-up period without treatment. The 2 adverse events (mild local itching and mild viral respiratory infection) were evaluated as not related to the tested medical device,

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proving its safety; patients recovered from symptoms after a few days and in both cases the investigator ruled out the suspicion of a BV recurrence.

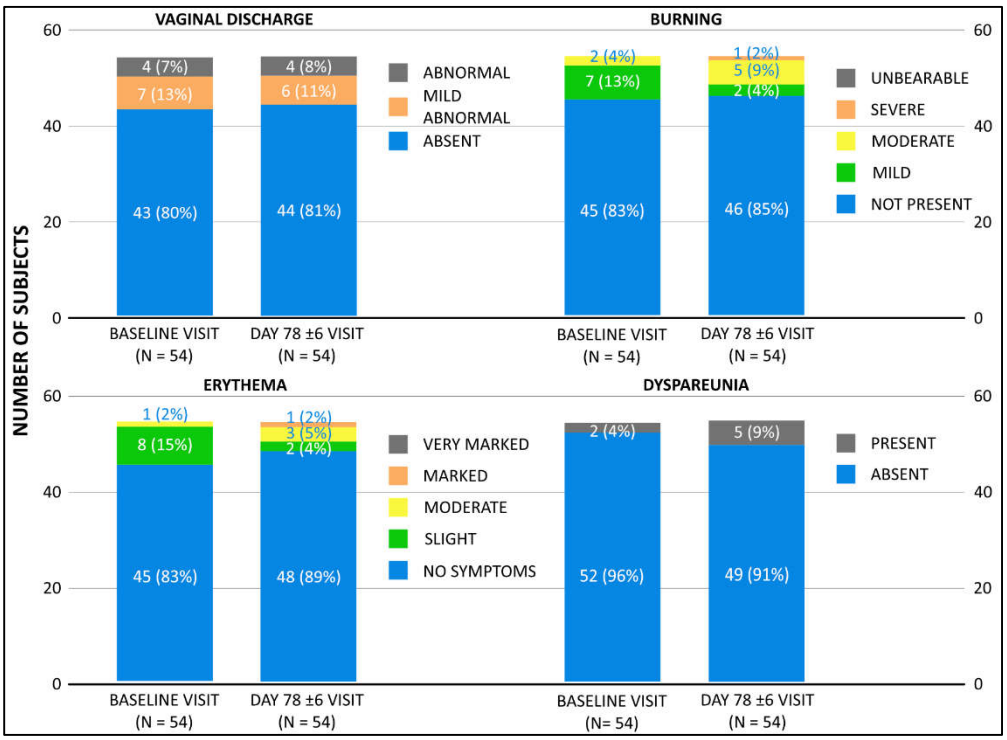


**Figure 6.** Vaginal discharge, burning, erythema, and dyspareunia from Baseline to day 78±6.

## 4. Discussion

BV treatment is usually effective as demonstrated by a reported cure rate of 80–90 % after one month, although a recurrence rate of about 30% is reported, as well [8]. Additionally, a BV recurrence rate of up to 80 % has been evidenced within 9 months [21]. Therefore, it is necessary for the scientific community to explore alternative methods of treatment for BV, since the current approach continues to be unsatisfactory for the prevention of recurrences.

This is the first study conducted to evaluate the clinical efficacy of PLGG vaginal ovules in the treatment of recurrent BV. Most women (34.28%) clinically cured after metronidazole vaginal treatment, confirming the results already shown in the interim analysis [17] and the following 3 cycles with PLGG, had no relapse of BV for all the 10 months of follow up without treatment. A wide range of factors, including sexual habits, smoking, and personal hygiene habits, can increase the vaginal pH, making conditions unfavourable for *Lactobacilli* and allowing the growth of predominantly anaerobic bacteria such as *Gardnerella vaginalis (GV)*, resulting in BV [22]. Some GV strains can produce a biofilm together with other bacteria, then providing protection from lactic acid and hydrogen peroxide and this phenomenon can lead to BV recurrence. Biofilm is a structured community of microorganisms in a self-produced extracellular matrix, adherent to the surface of epithelial cells [23,24]. Biofilm deficiencies are an important factor in the pathogenesis of BV, and they are not well managed by existing therapies. This situation can lead to inadequate treatment and can represent a potential determinant of recurrence.

The components of PLGG (polycarbophil, lauryl glucoside, glycerides) can have a synergistic action. Indeed, lauryl glucoside exerts a peculiar bacteriostatic action that can inhibit *Gardnerella vaginalis* growth. It was proven that *Gardnerella vaginalis* growth is reduced by contact with PLGG for 48 hrs [13]. Meanwhile, polycarbophil is a safe film-forming agent well known for its lack of toxicity, and lauryl glucoside is a non-ionic surfactant reinforcing the film-forming effect by reducing surface tension. We hypothesise that the

combination of these elements is the key to the peculiar mucoadhesive property of the product, able to impair the formation of the pathogen bacteria biofilm. Recurrence of BV may occur due to an inadequate restoration of a *Lactobacilli* dominant vaginal flora or because of ineffective treatment.

The absence of an untreated control group represents the main limitation and weakness of this study, but we considered unethical enrolling patients suffering from two or more episodes of recurrent BV as a control group.

The study was focused on demonstrating that the use of PLGG vaginal ovules in the treatment of BV not only reduces the rate of relapses, but also improves the microbiological parameters; this latter is shown by the positive effect on *Lactobacilli* in 66.7 % of treated cases.

## 5. Conclusions

The study supports the use of a specific vaginal product, that showed to possess a peculiar ability in creating and maintaining a protective vaginal biofilm that hinders the onset of BV and its recurrences. The study demonstrated that the short-term performance of PLGG can persist even 10 months overlong the end of PLGG treatment. Moreover, optimal profile of safety and tolerability of the PLGG treatment was shown as well.

**Supplementary Materials:** full trial protocol and database are available on request.

**Author Contributions:** conceptualization, FM; methodology, FM, and DFB; formal analysis, FM and SMR.; investigation, FM, PI, MB, DS, and CC; writing—original draft preparation, FM, SMR and DFB; writing—review and editing, FM, and DFB; visualization SMR; supervision, FM; project administration, DFB, MM, LIA, EC. All authors have read and agreed to the published version of the manuscript.

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**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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**Conflicts of Interest:** FM, PI, MB, DS, SMR, and CC declare no conflict of interest. DFB is employed at Opera CRO, the Contract Research Organization that managed the study, EC is a medical consultant for Italfarmaco SpA, and LIA and MM are employed at Italfarmaco SpA.

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