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The safety profile of probiotic VSL#3®. A meta-analysis of safety data from double-blind, randomized, placebo-controlled clinical trials

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Abstract. – OBJECTIVE: A high-concentration of a multi-strain probiotic mixture, VSL#3® is widely used 'whenever it is useful to promote the balance of intestinal flora'. As a food supplement, VSL#3® has been so far scarcely investigated on the aspect of safety. To fill this gap, in this paper, we analyzed the adverse events (AEs) recorded during the conduct of three (3) double-blind, randomized, placebo-controlled trials carried out to explore the efficacy of VSL#3® in various clinical settings. Data from a large open-label observational trial were also considered.

MATERIALS AND METHODS: All trials included in the analysis were carried out according to good clinical practice (GCP) rules. AEs were classified by System Organ Class (SOC), Preferred Term (PT) and frequency. Differences vs. placebo control were considered as statistically significant if the *p*-value was < 0.05.

RESULTS: A total of 120 patients were analyzed, 70 patients being included in the randomized controlled trials. In this population, 45 patients had at least one AE, 20 (64.5%) in the placebo group and 25 (64.1%) in the VSL#3® group. 29 patients had at least one related AE, 14 (45.2%) and 15 (38.5%) in the two treatment groups, respectively. Only one AE was assessed as serious, i.e., Foetal malformation, which occurred in the placebo group and was considered unrelated. No significant difference was found between VSL#3® and placebo for any of the SOC considered, with the exception of Injury, poisoning and procedural complications, which was in favor of VSL#3®.

CONCLUSIONS: Based on GCP-quality data from clinical trials, we conclude that VSL#3® is a safe and well-tolerated agent.

Key Words:
Probiotic, Metanalysis, System Organ Class, Adverse events.

Introduction

VSL#3® is a multi-strain probiotic mixture containing one strain of *Streptococcus*, three strains of Bifidobacteria and four strains of Lactobacilli[1]. VSL#3® is available in several oral formulations, including sachets containing 900 billion of colony-forming units (CFU), sachets containing 450 billion CFU and capsules containing 112.5 billion CFU.

VSL#3® meets the ESPGHAN criteria[2], according to which: "the probiotic microorganisms have to be present in a sufficient number by the end of the shelf-life, to pass through the gastrointestinal tract resisting acid and bile, to colonize the gut, and to retain functional properties required to obtain the suggested beneficial effect". VSL#3® showed very good resistance in *in vitro* models of gastric and intestinal juices, compared to other probiotics present on the Italian market[3].

VSL#3® is marketed as a food supplement and is widely used, with an estimated 80 million of doses sold every year in more than 40 countries worldwide. Its food supplement status does not permit claims of therapeutic indications with the same regulatory meaning of a licensed medicinal product, which can refer to specific pathological conditions where the product has been shown to be effective. Therefore, the indications for using VSL#3® are described in various ways in different countries. In most European Countries, including Italy, it is simply stated that VSL#3® should be used 'whenever it is useful to promote the balance of intestinal flora'. In the US, VSL#3® is a probiotic medical food "intended for the dietary

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management of Irritable Bowel Syndrome (IBS), Ulcerative Colitis (UC) or an ileal pouch".

A comprehensive class of medicinal products and food supplements, probiotics are commonly thought to be safe agents. Therefore, investigations on the safety profile of probiotics are a somewhat neglected issue. In this regard, probiotic medicinal products should take advantage of their regulatory status, since spontaneous reporting of adverse events (AEs) is a well-established and highly organized activity in the field of licensed drugs. Thus, in line of principle, less information is available for food supplements.

To fill the information gap concerning the safety profile of VSL#3®, in this paper we analyzed the AEs reported during the conduct of a number of clinical trials promoted by the Company (Actial Farmaceutica Srl, Rome, Italy) since 2016 to explore the effect of VSL#3® in different populations: obese pregnant women (ESDO trial), patients with UC (PROREM UC trial), women with osteoporosis (PROBONE trial) and with IBS (POST trial). We pooled and analyzed data from the ESDO, PROREM UC and PROBONE trials, because of close similarities in study design. Data from the POST trial were also considered but were analyzed in a separate setting, because of differences in study design that did not allow pooling of the data. All the trials included in this analysis were conducted according to the good clinical practice (GCP) rules, thereby generating high-quality safety data.

Materials and Methods

VSL#3® is a high-concentration multi-strain probiotic mix manufactured in Italy and containing: *i*) one strain of *Streptococcus thermophilus* BT01; *ii*) three strains of Bifidobacteria: *B. breve* BB02, *B. animalis* subsp. *lactis* BL03 (previously identified as *B. longum* BL03) and *B. animalis* subsp. *lactis* BI04 (previously identified as *B. infantis* BI04); *iii*) four strains of Lactobacilli: *L. acidophilus* BA05, *L. plantarum* BP06, *L. paracasei* BP07, and *L. helveticus* BD08 (previously identified as *L. delbrueckii* subsp. *bulgaricus BD08)*[1].

VSL#3® (Actial Farmaceutica Srl, Rome, Italy) has been used in four clinical studies in different populations: obese pregnant women (ESDO study, Italy), ulcerative colitis (PROREM UC study, Italy), women with osteoporosis (PROBONE study, US) and Irritable Bowel Syndrome (POST study, Italy) (Table I). In this paper we performed a pooled analysis of safety data deriving from these clinical studies. For the first three populations, data derived from randomized, placebo-controlled, clinical trials whereas, for the last population, data were gathered from an observational clinical study. All the studies included monitoring of AEs. All the present clinical trials have been sponsored directly by Actial Farmaceutica Srl or its subsidiaries.

All the studies have been approved by local Ethics Committees. The randomized, placebo-controlled, clinical trials were prematurely interrupted due to slow recruitment rate (ESDO study) or to administrative issues (PROBONE and PROREM UC studies). No study has been interrupted due to safety or clinical issues. The observational study was completed. Study design, populations and principal endpoints are described below.

ESDO Study

This was a single-center, randomized, double-blind, placebo-controlled, pilot study, to evaluate the effect of probiotic administration in obese pregnant women on body weight control, incidence of obstetric complications (gestational diabetes, preeclampsia), maternal vascular set-up and distribution of body water and maternal-fetal outcomes (Table I). Patients were also evaluated for adherence to treatment. Thirty (30) obese women were planned to be enrolled during pregnancy, between the 11^{th}-13^{th} weeks of gestation and randomized to receive VSL#3® (Lot. No. 610064) in sachets of 450 billion CFU or placebo (Lot. No. 610065), to be taken for 30 days. Twenty-one (21) patients were enrolled in the study, 12 in the active group (VSL#3® treatment) and 9 in the placebo group. There were no differences in the baseline characteristics of the two groups, who are also similar in terms of age and body mass index (BMI). Only 16 patients completed the 30-days follow-up (76.2%), eight in the active group and eight in the placebo group (Table II).

PROREM UC Study

This was a double-blind, randomized, placebo-controlled, single-center, dose-finding, pilot study evaluating the efficacy of VSL#3® in the maintenance of clinical and endoscopic remission of mild-to-moderate ulcerative colitis. The primary aim of this study was to evaluate the long-term efficacy of two different dosages of VSL#3® (Lot. No. 703094) added to standard maintenance

therapy with aminosalicylates (5-ASA) in an adult population of patients with mild-to-moderate UC in remission, compared with the standard therapy (5-ASA) plus placebo (Lot. No. 703096) (Table I). Two different oral doses of VSL#3® added to standard therapy (5-ASA) were investigated: 900 and 1800 billion bacteria per day. Patients were randomized in a 1:1:1 ratio to the following three arms:

- group A: mesalamine 2.4 g/day in once daily administration plus VSL#3® 450 billion CFU sachets, two sachets per day (900 billion bacteria per day) for 12 months;
- group B: mesalamine 2.4 g/day in once daily administration plus VSL#3® 450 billion CFU sachets, two sachets twice a day (1800 billion bacteria per day) for 12 months;
- group C: mesalamine 2.4 g/day in once daily administration plus placebo for 12 months.

Thirty-nine (39) patients with a history of mild-to-moderate ulcerative colitis and clinical and endoscopic remission in maintenance therapy with 5-ASA during screening period were planned to be enrolled.

The study was prematurely interrupted, after having randomized 14 patients, but four patients had no post-baseline evaluation. So, ten (10) patients completed the study: four in group A, three in group B and three in group C, respectively (Table II).

Considering the whole sample, age ranged between 29 and 70 years with a mean (SD) value of 48 (11.3) years; 35.7% of patients were male. Forty-four percent (44%) of patients reported a diagnosis of left-sided colitis, 55.5% extensive colitis, and for one patient the information was missing. The median time of follow-up was eighteen months.

PROBONE Study

This was a randomized, double-blind, placebo-controlled, pilot clinical study to evaluate the efficacy on bone mineral density, bone turnover of the probiotic VSL#3® in healthy early postmenopausal women. The aim of this study was to evaluate if dietary supplementation with VSL#3® twice daily for 12 months has any effect on bone mineral density (BMD), inflammation, or meta-

Table I. Main study characteristic.

Disease under study:	
ESDO	Obese pregnant women.
PROREM UC	Mild to moderate ulcerative colitis.
PROBONE	Healthy early postmenopausal women.
POST	Irritable bowel disease.
Primary outcome:	
ESDO	Peripheral vascular resistance, maternal foetal outcome.
PROREM UC	Maintenance of clinical & endoscopic remission.
PROBONE	Bone mineral density, bone turnover.
POST	Compliance to prescription, factors influencing compliance.
Study design:	
ESDO	Double-blind, randomized, placebo-controlled study.
PROREM UC	Double-blind, randomized, placebo-controlled, dose-finding study.
PROBONE	Double-blind, randomized, placebo-controlled study.
POST	Observational, prospective, uncontrolled study.
Treatment duration:	
ESDO	30 days.
PROREM UC	12 months.
PROBONE	12 months.
POST	From 2 to 8 weeks..
Treatment dose:	
ESDO	450 billion CFU.
PROREM UC	900-1800 billion CFU.
PROBONE	450 billion CFU.
POST	450-900 billion CFU.

The table lists the main characteristics of the studies included in the paper and in particular: Disease under study, Primary outcome, Study design, Treatment duration, Treatment dose.

bolic and endocrine markers in postmenopausal women with osteopenia. The primary endpoint was to assess changes in BMD as measured by dual x-ray absorpiometry (DEXA) at lumbar spine after an intervention period of 12 months. Changes in BMD at the femoral neck and total hip area at 12 months were also measured along with changes in bone turnover markers during the period of 12 months (Table I). Twenty (20) patients per group were initially planned to be randomized to receive:

- 500 mg of calcium carbonate or calcium citrate per day in a single dose, 1000 U of vitamin D_3 per day in a single dose and VSL#3® (450 billions of bacteria) per day in a single dose (active group).
- 500 mg of calcium carbonate or calcium citrate per day in a single dose, 1000 U of vitamin D_3 per day in a single dose, and placebo per day in a single dose (control group).

Thirty-five (35) patients were enrolled and randomized: 18 patients in the active group (Lot. No. 610064, 703093, 709002, 802112) and 17 in the placebo group (Lot. No. 610065, 703095, 709003, 802113), respectively. Four (4) patients did not complete the study: three in VSL#3® group, because they were lost to follow-up and one patient in the placebo group, because of consent withdrawal. The median time of follow-up was more than nine months (Table II).

POST Study

This was a single-center, observational, prospective study to evaluate the compliance to the prescription of probiotic therapy in real life and to identify factors able to influence adherence to therapy in patients with Irritable Bowel Syndrome[4] (Table I).

Fifty (50) consecutive patients diagnosed with IBS according to Rome IV criteria and receiving a clinical prescription of VSL#3® for their IBS symptoms were evaluated for eligibility. Patients have been enrolled from January 2018 until December 2018. After enrollment, they received a diary at the beginning of the therapy in order to evaluate adherence, safety and effect of treatment and after two months, during a face to face visit or a phone call, patients were evaluated for adherence, AEs and subjective relief of symptoms (Table I). Fifty (50) patients (mean age 41± SD 14.4 years, 26% males) were enrolled as planned in the protocol and 49 completed the planned follow up (Table II). IBS subtypes are distributed as following: 44% diarrhea, 42% constipation and mixed in the remaining cases. Eighty-six percent (86%) of patients received a 4-week prescription of one sachet per day. The other patients received a prescription of one sachet per day for 2 weeks (6%), one sachet per day for 8 weeks (4%), two sachets per day for 2 weeks (2%) and one sachet per day for 15 days (2%).

Statistical Analysis

All analyses have been performed on the population of the subjects who were enrolled in the studies and who received at least one dose of the study treatment.

A descriptive analysis of demographic variables and the observation period has been performed two times, including and excluding the POST study. The treatment comparisons were carried out by means of the Student's *t*-test for age, Chi-square test for gender, and Mann Whitney test for the observation period.

Percentages were computed on a per-patient basis, i.e., patients with more than one AE were counted only once. This criterion was applied at each classification level and this is the reason why cells at the Preferred Term (PT) level do not sum up to cells at System Organ Class (SOC) level and the latter cells do not sum up to the counts at overall study level.

The number and percentage of subjects with at least one AE was presented at overall level and by SOC and PT, separately for patients treated with VSL#3® or placebo. Chi-Square or Fisher's exact tests were used to compare the incidences of AEs at the SOC level in VSL#3® and placebo-treated patients. Percentages of AEs including the POST study were calculated by dividing the number of patients with event by patient-months, calculated considering the time of follow-up of each patient.

AEs were defined related if the relationship with study treatment was considered possible, probable, or definitive by the Investigators. SAS software was used for all analyses. A p-value <0.05 is considered indicative of a statistically significant difference.

Results

A total of 120 patients has been evaluated, 31 treated with placebo and 89 with VSL#3®. The patient characteristics at baseline are summarized in Table II. There appears to be a good balance between the two treatment groups by age (overall mean value is 40.1), but not by gender (93.5% *vs.*

Table II. Patient characteristics.

	Total	Placebo	VSL#3®	*p*-value
Number of enrolled pts (Completed pts):				
Pooled Dataset	120 (106)	31 (27)	89 (79)	
Pooled without POST	**70 (57)**	**31(27)**	**39 (30)**	
ESDO	21 (16)	9 (8)	12 (8)	
PROREM UC	14 (10)	5 (3)	9 (7)	
PROBONE	35 (31)	17 (16)	18 (15)	
POST	50 (49)	-	50 (49)	
Age in years (Mean (SD)):				
Pooled Dataset	40.1 (13.1)	37.3 (10.2)	40.7 (13.6)	0.530
Pooled without POST	**38.9 (11.0)**	**37.3 (10.2)**	**40.0 (11.6)**	**0.567**
ESDO	32.9 (5.2)	33.0 (4.9)	32.8 (5.6)	
PROREM UC	48.0 (11.3)	45.2 (13.0)	49.5 (10.7)	
PROBONE	NA	NA	NA	
POST	41.0 (14.4)	-	41.0 (14.4)	
Gender (% Females):				
Pooled Dataset	65%	93.5%	55.1%	<0.001
Pooled without POST	**92.9%**	**93.5%**	**92.3%**	**0.841**
ESDO	100%	100%	100%	
PROREM UC	64.3%	60.0%	66.7%	
PROBONE	100%	100%	100%	
POST	74%	-	74%	
Observation period in days (Median):				
Pooled Dataset	56.0	199.0	56.0	0.006
Pooled without POST	**197.0**	**199.0**	**195.0**	**0.985**
ESDO	30.0	30.0	30.0	
PROREM UC	545.0	545.0	545.0	
PROBONE	287.0	329.0	244.5	
POST	56.0	-	56.0	

The table lists main characteristics of patients included in the different clinical trials. Patients are stratified by treatment (placebo or VSL#3). Data summaries are shown both including all the four trials and including only the randomized controlled trials (POST is excluded). SD means standard deviation. A p-value <0.05 is considered indicative of a statistically significant difference.

55.1% of females in placebo and VSL#3®, respectively; $p<0.001$) and by observation period (median is 199 days *vs.* 56 days in the two treatment groups, respectively; $p=0.006$). The difference in the observation period between the two treatments is especially problematic, because it would bias all treatment comparisons on the incidence of AEs, unless a statistical adjustment is applied. The imbalance is generated by the POST study, which had no control group. When this study is removed from the statistical analysis, the two treatment groups appear well balanced with respect to all considered factors, as shown in Table II. We decided, therefore, to present the main results on AEs by excluding the POST study, therefore considering a sample of 70 patients, 31 on placebo and 39 on VSL#3®. However, it should be considered that, while we excluded the POST data for the sake of optimizing study design, the conclusions on the safety profile of VLS#3® do not change upon including the POST study and adjusting the imbalance in observation time by using patient-months instead of patients as denominators (data presented in **Supplementary Table S1**).

The percentage of patients with at least one AE is presented in Table III by SOC and PT. There are in total 45 patients with at least one AE, 20 (64.5%) in the placebo group and 25 (64.1%) in the VSL#3® group, and 29 patients with at least one related AE, 14 (45.2%) and 15 (38.5%) in the two treatment groups, respectively. The vast majority of the related AEs belong to the *Gastrointestinal disorders* SOC and actually only 6 patients (4 treated with placebo and 2 with VSL#3®) experienced related AEs other than gastrointestinal (the concerned SOCs are Infections and infestations, Investigations, and Nervous System disorders).

Table III. Patients with at least one AE by SOC and PT (without POST study). Patients with related AEs are reported in bold.

System Organ Class (SOC)	Preferred Term (PT)	Total (N=70) n (%)	Placebo (N=31) n (%)	VSL#3® (N=39) n (%)	*p*-value
Patients with at least one AE:					
Any AEs		45 (64.3%)	20 (64.5%)	25 (64.1%)	0.971
Related AEs		**29 (41.4%)**	**14 (45.2%)**	**15 (38.5%)**	**0.572**
Blood and lymphatic system disorders		1 (1.4%)	1 (3.2%)	0	0.443
	Iron deficiency anemia	1 (1.4%)	1 (3.2%)	0	
Cardiac disorders		1 (1.4%)		1 (2.6%)	1.000
	Palpitations	1 (1.4%)		1 (2.6%)	
Congenital, familial and genetic disorders		1 (1.4%)	1 (3.2%)	0	0.443
	Fetal malformation	1 (1.4%)	1 (3.2%)	0	
Ear and labyrinth disorders		1 (1.4%)		1 (2.6%)	1.000
	Vertigo	1 (1.4%)		1 (2.6%)	
Eye disorders		1 (1.4%)	1 (3.2%)	0	0.443
	Asthenopia	1 (1.4%)	1 (3.2%)	0	
	Vision blurred	1 (1.4%)	1 (3.2%)	0	
Gastrointestinal disorders See Table IV for details		35 (50%)	16 (51.6%)	19 (48.7%)	0.810
General disorders and administration site conditions			10 (14.3%)	5 (16.1%)	5 (12.8%) 0.694
	Asthenia (*)	9 (12.9%)	5 (16.1%)	4 (10.3%)	
	Influenza like illness	2 (2.9%)	0	2 (5.1%)	
	Vaccination site pain	1 (1.4%)	1 (3.2%)	0	
Immune system disorders		1 (1.4%)	1 (3.2%)	0	0.443
	Seasonal allergy	1 (1.4%)	1 (3.2%)	0	
Infections and infestations:					
Any AEs		14 (20%)	8 (25.8%)	6 (15.4%)	0.279
Related AEs		**3 (4.3%)**	**2 (6.5%)**	**1 (2.6%)**	**0.580**
	Ear infection	1 (1.4%)	0	1 (2.6%)	
	Fungal skin infection	1 (1.4%)	1 (3.2%)	0	
	Gastroenteritis (*):				
	Any AEs	3 (4.3%)	2 (6.5%)	1 (2.6%)	
	Related	**3 (4.3%)**	**2 (6.5%)**	**1 (2.6%)**	
	Herpes zoster	1 (1.4%)	0	1 (2.6%)	
	Hordeolum	1 (1.4%)	0	1 (2.6%)	
	Influenza (*)	7 (10.0%)	4 (12.9%)	3 (7.7%)	
	Urinary tract infection (*)	4 (5.7%)	2 (6.5%)	2 (5.1%)	
	Vaginal infection	1 (1.4%)	1 (3.2%)	0	
Injury, poisoning and procedural complications		4 (5.7%)	4 (12.9%)	0	0.034
	Foot injury	1 (1.4%)	1 (3.2%)	0	
	Insect bite NOS	1 (1.4%)	1 (3.2%)	0	
	Muscle strain	1 (1.4%)	1 (3.2%)	0	
	Respiratory fume inhalation disorder	1 (1.4%)	1 (3.2%)	0	
Investigations:					
Any AEs		2 (2.9%)	2 (6.5%)	0	0.193
Related AEs		**1 (1.4%)**	**1 (3.2%)**	**0**	**0.443**
	Blood pressure increased	1 (1.4%)	1 (3.2%)	0	
	Weight increased:				
	Any AEs	1 (1.4%)	1 (3.2%)	0	
	Related	**1 (1.4%)**	**1 (3.2%)**	**0**	

Table continued

Table III. *(Continued).* Patients with at least one AE by SOC and PT (without POST study). Patients with related AEs are reported in bold.

System Organ Class (SOC)	Preferred Term (PT)	Total (N=70) n (%)	Placebo (N=31) n (%)	VSL#3® (N=39) n (%)	*p*-value
Musculoskeletal and connective tissue disorders		17 (24.3%)	8 (25.8%)	9 (23.1%)	0.791
	Arthralgia (*)	4 (5.7%)	1 (3.2%)	3 (7.7%)	
	Joint stiffness	1 (1.4%)	0	1 (2.6%)	
	Musculoskeletal pain (*)	16 (22.9%)	8 (25.8%)	8 (20.5%)	
Nervous system disorders:					
Any AEs		17 (24.3%)	10 (32.3%)	7 (17.9%)	0.165
Related AEs		**2 (2.9%)**	**1 (3.2%)**	**1 (2.6%)**	1.00
	Dizziness	2 (2.9%)	1 (3.2%)	1 (2.6%)	
	Headache (*):				
	Any AEs	15 (21.4%)	9 (29.0%)	6 (15.4%)	
	Related	**2 (2.9%)**	**1 (3.2%)**	**1 (2.6%)**	
Pregnancy, puerperium and perinatal conditions		1 (1.4%)	0	1 (2.6%)	1.000
	Hyperemesis gravidarum	1 (1.4%)	0	1 (2.6%)	
Psychiatric disorders		1 (1.4%)	1 (3.2%)	0	0.443
	Insomnia	1 (1.4%)	1 (3.2%)	0	
Renal and urinary disorders		1 (1.4%)	0	1 (2.6%)	1.000
	Dysuria	1 (1.4%)	0	1 (2.6%)	
Respiratory, thoracic and mediastinal disorders		8 (11.4%)	3 (9.7%)	5 (12.8%)	1.000
	Dyspnea	1 (1.4%)	1 (3.2%)	0	
	Nasal congestion (*)	4 (5.7%)	1 (3.2%)	3 (7.7%)	
	Oropharyngeal pain	5 (7.1%)	2 (6.5%)	3 (7.7%)	
	Sinus congestion (*)	2 (2.9%)	0	2 (5.1%)	
Skin and subcutaneous tissue disorders		8 (11.4%)	5 (16.1%)	3 (7.7%)	0.452
	Dry skin	2 (2.9%)	2 (6.5%)	0	
	Eczema	1 (1.4%)	1 (3.2%)	0	
	Photosensitivity reaction	1 (1.4%)	1 (3.2%)	0	
	Pruritus	2 (2.9%)	1 (3.2%)	1 (2.6%)	
	Rash	1 (1.4%)	1 (3.2%)	0	
	Rash pruritic	2 (2.9%)	0	2 (5.1%)	
Surgical and medical procedures		3 (4.3%)	0	3 (7.7%)	0.249
	Antibiotic therapy	3 (4.3%)	0	3 (7.7%)	

Percentages were computed on a per-patient basis. Related AEs are those AEs which were considered as possibly, probably, or definitively related to the study treatment by the Investigators. When there is no split between Any and Related AEs, it means that there was no related AE. A *p*-value <0.05 is considered indicative of a statistically significant difference.

(*) Some PTs have been combined as follows: asthenia includes asthenia & fatigue; gastroenteritis includes diarrhoea & infectious gastroenteritis; influenza includes influenza & nasopharyngitis; urinary tract infection includes cystitis & urinary tract infection; arthralgia includes arthralgia & arthritis; muscoloskeletal pain includes back pain, bone pain, flank pain, musculoskeletal pain, myalgia, neckpain & pain in extremity; headache includes headache, migraine & sinus headache; nasal congestion includes nasal congestion, nose congestion & sneezing; sinus congestion includes sinus congestion & Sinus disorder.

Only one AE was assessed as serious, i.e., *Foetal malformation*, which occurred in the placebo group of the ESDO study and was considered unrelated.

Considering all reported AEs, the most represented SOCs are: (1) *Gastrointestinal disorders* (50% in the total sample, 51.6% in placebo and 48.7% in VSL#3®), followed by (2) *Nervous system disorders* (32.3% in placebo and 17.9% in VSL#3®), (3) *Musculoskeletal and connective tissue disorders* (25.8% in placebo and 23.1% in VSL#3®), (4) *Infection and infestations* (25.8% in placebo and 15.4% in VSL#3®), (5) *General disorders and administration site conditions* (14.3%),

Table IV. Patients with at least one AE in the Gastrointestinal Disorders SOC (without POST study).

Preferred Term (PT)	Total (N=70) n (%)	Placebo (N=31) n (%)	VSL#3® (N=39) n (%)	*p*-value
Whole SOC:				
Any AEs	35 (50%)	16 (51.6%)	19 (48.7%)	0.810
Related AEs	29 (41.4%)	14 (45.2%)	15 (38.5%)	0.572
Abdominal distension:				
Any AEs	5 (7.1%)	4 (12.9%)	1 (2.6%)	
Related AEs	5 (7.1%)	4 (12.9%)	1 (2.6%)	
Colitis ulcerative:				
Any AEs	1 (1.4%)	0	1 (2.6%)	
Related AEs	0	0	0	
Constipation: *(*)*				
Any AEs	10 (14.3%)	5 (16.1%)	5 (12.8%)	
Related AEs	6 (8.6%)	3 (9.7%)	3 (7.7%)	
Diarrhoea: *(*)*				
Any AEs	16 (22.9%)	9 (29.0%)	7 (17.9%)	
Related AEs	15 (21.4%)	8 (25.8%)	7 (17.9%)	
Dyspepsia: (*)				
Any AEs	17 (24.3%)	8 (25.8%)	9 (23.1%)	
Related AEs	13 (18.6%)	6 (19.4%)	7(17.9%)	
Flatulence:				
Any AEs	10 (14.3%)	5 (16.1%)	5 (12.8%)	
Related AEs	10 (14.3%)	5 (16.1%)	5 (12.8%)	
Food poisoning:				
Any AEs	1 (1.4%)	0	1 (2.6%)	
Related AEs	0	0	0	
Gastrointestinal disorders: (*)				
Any AEs	9 (12.9%)	5 (16.1%)	4 (10.3%)	
Related AEs	8 (11.4%)	5 (16.1%)	3 (7.7%)	
Nausea:				
Any AEs	7 (10.0%)	4 (12.9%)	3 (7.7%)	
Related AEs	3 (4.3%)	2 (6.5%)	1(2.6%)	
Rectal haemorrhage:				
Any AEs	1 (1.4%)	0	1 (2.6%)	
Related AEs	0	0	0	
Vomiting:				
Any AEs	5 (7.1%)	2 (6.5%)	3 (7.7%)	
Related AEs	4 (5.7%)	2 (6.5%)	2 (5.1%)	

Percentages were computed on a per-patient basis. Related AEs are those AEs which were considered as possibly, probably, or definitively related to the study treatment by the Investigators. A *p*-value <0.05 is considered indicative of a statistically significant difference.
(*) Some PTs have been combined as follows: Constipation includes Constipation, Dyschezia, Faeces hard & Stools hard; Diarrohea includes Defaecation urgency, Diarrhorea, Faeces soft; Dyspesia includes Dyspepsia, Eructation, Reflux gastritis & Regurgitation; Gastrointestinal disorders includes Abdominal discomfort, Abdominal pain, Defaecation disorder, Faeces discoloured, Frequent bowel movements, Gastrointestinal disorders, Gastrointestinal motility disorder, Gastrointestinal pain & Gastrointestinal sounds abnormal.

and (6) *Respiratory, thoracic and mediastinal disorders* and *Skin and subcutaneous tissue disorders* (both SOCs, reaching overall a frequency of 11.4%). All other SOCs have percentages around or below 5%. Among the SOCs with a frequency higher than 5%, the comparisons between treatment groups are slightly in favor of VLS#3® in all cases but the *Respiratory, thoracic and mediastinal disorders* SOC. No treatment difference at SOC level is statistically significant except for *Injury, poisoning and procedural complications*: this difference is in favor of VSL#3® (percentages were 12.9% among the placebo-treated patients and zero among the VSL#3® treated ones).

Gastrointestinal disorders are the most relevant SOC for VSL#3®, given its indications. These AEs are illustrated in Table IV. The incidence of related AEs appears to be lower in VSL#3® than in placebo for all the PTs under this SOC: the most represented related PTs are Dyspepsia [6 (19.4%) and 7 (17.9%) in the placebo and VSL#3® groups, respectively] and Diarrhea [8 (25.8%) and 7 (17.9%) in the same two groups]. The results in terms of all reported AEs are similar.

Placebo and VSL#3® groups appear well balanced as for their AE profile even when stratifying the results by age (up to 58 years and above). Data were also stratified by gender and dose (one or two sachets per day *vs.* four sachets per day), but the number of male patients and patients treated with the high dose are too low to draw any conclusion (data presented in **Supplementary Table S2**).

Discussion

From the analysis of the described study data, it emerges that VSL#3® shows an overall favorable safety profile: no statistically significant difference between VSL#3® and placebo-treated groups was found (regardless of whether the AEs were assessed as related or not to the study treatment) for any of the SOCs considered, with the single exception of *Injury, poisoning and procedural complications* (which, however, was in favor of VSL#3®). Apart from the statistical significance, in almost all SOCs the percentages of patients with AEs were slightly higher in placebo than in VSL#3®, showing that the observed AEs were likely a manifestation of the background disease. The favorable safety profile of VSL#3® was confirmed when including in the analysis the data from the POST trial. It is also worthy of note that, apart from the ESDO trial, the exposure to the investigational agent or to placebo had a long-term (12 months) duration. In order to compare the safety profile of VSL#3® with those of similar probiotic agents, a relevant source of information is represented by the summaries of product characteristics (SmPCs) of probiotic medical products. We analyzed the SmPCs of 8 probiotic medicinal products, available in the databank of the *Agenzia Italiana del Farmaco* (AIFA), looking at the section 4.8 (Undesirable effects). Three (3) products – Enterogermina® (Sanofi S.p.a., Milan, Italy), Eptavis/Yovis® (Alfasigma S.p.a., Bologna, Italy) and Codex® (Zambon Italia S.p.a., Milan, Italy) – describe AEs in a structured form, i.e., provide a description by SOC, PT and frequency of events. Enterogermina® reports *Skin and subcutaneous tissue disorders* (hypersensitivity reactions, including rash, urticaria and angioedema) and *Infections and infestations* (bacteraemia, in immunocompromised patients), both with unknown frequency. Eptavis® and Yovis® report *Gastrointestinal disorders* (constipation and abdominal pain) as uncommon (less than 0.1%) and *Skin and subcutaneous tissue disorders* (urticaria and itching) with unknown frequency. Codex® reports *Gastrointestinal disorders* (flatulence) as rare (between 0.1 and 0.01%), and *Skin and subcutaneous tissue disorders* (hypersensitivity reactions, including angioedema, itching, urticaria and localized or systemic rash), *Immune system disorders* (anaphylactic reactions or shock) and *Infections and infestations* (fungemia in critically-ill or immunocompromised patients) as very rare (less than 0.01%). All other products describe AEs in a narrative form. Biogermin® (Union Health S.r.l., Chieti, Italy) states that 'no undesirable effect has ever been reported using the drug'. Bioflorin® (Sanofi S.p.a., Milan, Italy) reports that 'so far no side effects have been reported as an effect of treatment'. Lacteol® (Bruschettini S.r.l., Genova, Italy) states that 'undesirable effects are not known'. Infloran® (Laboratorio Farmaceutico SIT, Pavia, Italy) states that 'at recommended dosages, no undesirable effects have been reported'. Eventually, Endolac® (Proge Farm S.r.l., Novara, Italy) and Morelac® (Ipsen Consumer Healthcare S.r.l., Milan, Italy) declare that 'there is no report in the literature of undesirable effects due to the medicine'.

It is understood that the data presented here are not directly comparable with those reported in the SmPCs of probiotics, for a number of reasons: *i*) the information included in SmPCs derives from clinical trials as well as from spontaneous reporting and data from the literature; such different sources of information do not share the same lev-

el of certified quality. Moreover, the total number of patients exposed to the drug is ill-defined or even unknown, making it difficult to obtain a correct estimate of the frequency of AEs reported in SmPCs; *ii*) SmPCs report related AEs only, since the description of both related and unrelated events is deemed unnecessary. In addition, in case the placebo group is shown to have the same AE profile of the drug, this is specified in the SmPC. Therefore, a proper comparison between our data and those reported in the SmPCs should consider only related AEs with the specification that the same events were observed in the placebo group; *iii*) as mentioned before, there is no homogeneity about the quality of AEs reporting in the SmPCs; therefore, it is difficult to compare each SOC and PT presented in this manuscript with generic AEs qualitative descriptions. With these limitations in mind, nevertheless, we can conclude that the profile of safety of VSL#3®, as it emerged in the present study, is broadly comparable to those of similar probiotic medicinal products.

Another relevant source of information, i.e., the literature concerning the safety of probiotics, is altogether poor, probably because most AEs related to the use of probiotics are not deemed worth reporting. We found a cluster of reports concerning several cases of fungemia associated to the use of *S. Boulardii*[5-8]. These cases were usually observed in critically ill or immunocompromised patients and are correctly reported in the SmPCs of probiotics containing this strain. A highly quoted paper on the risks of probiotic use is the PROPATRIA trial[9]. This study was carried out in patients with acute pancreatitis and showed an increase in mortality in the group treated with the probiotic (a mixture of 2 *Bifidobacteria* and 4 *Lactobacilli* strains marketed in the Netherlands), raising doubts about the opportunity to use probiotics in critically ill patients. Recently, van den Nieuwboer and Claassen reviewed the issue of probiotic safety, including a thorough analysis of PROPATRIA study[10]. These authors conclude that probiotics are an overall safe class of products. If any discussion remains which concerns the issue of probiotic safety, this is due to various reasons, including the current need to increase reporting of (good quality) safety data[10]; the present work goes in the direction recommended by these authors.

Conclusions

In this paper, we analyzed the safety data collected during the conduct of four clinical trials investigating the effect of VSL#3® in various clinical conditions. Three of these studies were randomized controlled trials comparing VSL#3® with placebo. All the trials were conducted according to the GCP rules. We showed that the safety profile of VSL#3® is not statistically different compared to that of placebo. We discussed our findings within the framework of the information available on the safety profile of probiotics; the present data confirm the overall notion that probiotics as a class are safe agents. Consequently, we can also conclude that the profile of safety of VSL#3®, as it emerged in the present study, is broadly comparable to those of similar probiotic medicinal products.

Conflict of Interests

Authors' declaration of personal interests: Antonella Bacchieri and Pierluigi Navarra have served as a consultant for Actial Farmaceutica.

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