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Randomised clinical trial: *Bifidobacterium bifidum* MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life: a double-blind, placebo-controlled study

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Abstract

Background. Recent research suggests that an imbalance of the intestinal microbiota and a dysfunctional intestinal barrier might trigger irritable bowel syndrome (IBS). As probiotics have been reported to restore the intestinal microbiota and the gut barrier, the therapeutic potential of probiotics within IBS became of strong interest. Aim. To assess the efficacy of *Bifidobacterium bifidum* MIMBb75 in IBS. Methods. 122 patients were randomized to receive either placebo (N=62) or MIMBb75 (N=60) once a day for four weeks. The severity of IBS symptoms was recorded daily on a 7 point Likert scale. Results. MIMBb75 significantly reduced the global assessment of IBS symptoms by -0.88 points [95%CI: -1.07; -0.69] as compared to only -0.16 [95%CI: -0.32; 0.00] points in the placebo group (p<0.0001). MIMBb75 also significantly improved the IBS symptoms pain/discomfort, distension/bloating, urgency and digestive disorder. The evaluation of the SF12 sum scores showed a significant gain in quality of life within the bifidobacteria group. Furthermore, adequate relief was reported by 46.7% of the patients in the bifidobacteria and only by 11.3% of the patients in the placebo group (p<0.0001). Overall responder rates were 56.7% in the bifidobacteria group but only 21.0% in the placebo group (p=0.0001). MIMBb75 was well tolerated and adverse events were not different from placebo. Conclusion. *B. bifidum* MIMBb75 effectively alleviates global IBS and improves IBS symptoms simultaneously with an improvement of quality of life. Considering the high efficacy of MIMBb75 in IBS along with the good side effect profile, MIMBb75 is a promising candidate for IBS therapy.

Keywords: Irritable Bowel Syndrome, IBS, Colon irritable, Intestinal microbiota, Probiotics, Bifidobacteria

1. Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal functional disorder, diagnosed through the Rome III criteria (1). Abdominal pain, flatulence and bloating are common IBS symptoms for which no endoscopic, biochemical or radiological cause is verifiable. IBS affects 15-20 % of the population, with a highly increasing tendency in industrial nations (2, 3, 4). The pathophysiology of IBS is yet only partly understood. Recent research suggests that an imbalance of the intestinal microbiota with a significant reduction of bifidobacteria and a dysfunctional intestinal barrier with subsequent bacterial translocation may contribute to the development of IBS and its symptoms (5, 6, 7, 8, 9).

As probiotics have been reported in several studies to restore the intestinal microbiota and gut barrier as well as hinder bacterial translocation, the therapeutic potential of probiotics in IBS has become of strong interest (10, 11). However the efficacy of probiotics is strongly strain specific and only certain strains might be able to improve IBS and its symptoms (12, 13, 14). The ability of specific strains to adhere well to intestinal cells may play a pivotal role in altering the intestinal microbiota and increasing the intestinal barrier, which might be of significant value especially in the treatment of IBS. Guglielmetti et al. were able to show in a Caco-2 cell line that the adherence of *B. bifidum* MIMBb75, which was isolated from a fecal sample of a healthy adult, was significantly better than that of well-studied commercial probiotics (15, 16). Taking into consideration the pathophysiology of IBS and the role of adherence of probiotics regarding the restoration of the intestinal microbiota as well as of the gut barrier, *B. bifidum* MIMBb75 might be effective for IBS treatment. As the conventional medical treatment of IBS is unsatisfactory, we have evaluated the efficacy of *B. bifidum* MIMBb75 in IBS in a double blind, placebo-controlled multi-center study.

2. Patients and Methods

2.1 Study Population

Patients were recruited in several physician centers in Bavaria, Germany from principal investigators and by advertisements. The nutritional study protocol has been presented to the Ethics Committee of the Bavarian Chamber of Physicians. For inclusion, subjects aged between 18 and 68 years with mild to moderate IBS (Rome III criteria) have been considered. Individuals with inflammatory organic gastrointestinal disease, systemic diseases, cancer, autoimmune diseases, diabetes, known lactose intolerance or immunodeficiency, known further abdominal surgery except appendectomy, being older than 50 years and having had a positive sigmoidoscopy or coloscopy within the last five years, diagnosed hyperthyroidism, use of antipsychotics or systemic corticosteroids for at least 3 months prior to study start, major psychiatric disorder, celiac disease or pregnancy had been excluded.

2.2 Study Design

This study was performed as a prospective, multi-center, randomized, double-blind, placebo-controlled, two-arm nutritional study.

Throughout the study, patients recorded their global IBS symptoms on a daily basis as well as individual IBS symptoms using a patient diary. Additionally, patients have been questioned at a physician site for IBS symptoms (visit 2-4) and quality of life (visit 3 and 4).

Physician visits took place at screening, after two weeks (run-in phase), after 6 weeks (end of treatment) and after 8 weeks (end of wash-out phase) (Figure 1).

After the patients have given their written informed consent, they qualified for the screening examination on day 1 (visit 1), which included a complete medical history and physical examination. A blood sample was taken for analysis in a central laboratory, including a pregnancy test. At the screening visit patients were instructed to maintain their eating and life style habits throughout the study. A patient diary was handed out.

At the second visit (day 15) diaries were reviewed. Patients who had at least 2 days with mild to moderate pain during the second week of run-in and who fulfilled all inclusion criteria and who did not violate any of the exclusion criteria were 1:1 randomized to receive either *B. bifidum* MIMBb75 or placebo. The treatment was allocated according to a computer-generated blocked randomization list with a block size of 4. The block size was not disclosed to the investigators. During the intervention period, patients received either one probiotic capsule daily over a 4 week period or an identical appearing placebo. The allocation was blinded to both patients and site staff.

At the end of the treatment phase (visit 3, day 43), investigators collected the unused study product and empty sachets in order to confirm compliance. Diaries were collected and reviewed.

After the nutritional supplement-free wash-out phase (visit 4, day 57), a complete physical examination was performed and a blood sample was taken.

Bisacodyl and Loperamid were allowed as rescue medication. Other probiotics and medications that might influence the efficacy of the study product were not allowed.

2.3 Probiotic Preparation

Bifidobacterium bifidum MIMBb75 was isolated from the fecal sample of a healthy adult. *Bifidobacterium bifidum* is a species that is commonly detected in the feces of

healthy adults and infants. Several strains of *B. bifidum* have been observed to adhere very well to human intestinal cell lines. In a human Caco-2 model, the adhesion ability of *B. bifidum* MIMBb75 was even significantly better than that of well-studied commercial probiotics.

Guglielmetti et al. showed that BopA, a surface protein, which functions as a protein promoter, is directly involved in the adhesion to Caco-2 cells (15). BopA has been found in several strong adhesive *B. bifidum* strains, indicating that BopA might be an indicator of strong adherence. Additionally *B. bifidum* MIMBb75 showed considerably more adhesion to the Caco-2 monolayer in the presence of fucose and mannose and less when *B. bifidum* MIMBb75 grew in Oxgall bile salts compared to standard environmental conditions. The colonization strategy of this bacterium could be influenced by several factors varying along the gastrointestinal tract, such as the presence of specific sugars and bile salts and the pH, likely supporting adhesion of B. bifidum MIMBb75 to distal sites of the gut (16).

Nutritional supplement was prepared under good manufacturing process (GMP) conditions. *B. bifidum* MIMBb75 was grown in a protein-rich liquid growth medium, harvested through centrifugation, stabilized, freeze-dried, milled and sieved. The dry powder bacteria were mixed with an excipient and filled into uncoated capsules of 1x 10^9 cfu. Placebo capsules appeared identical and contained maltodextrin.

2.4 Endpoint definitions

The prospectively defined primary efficacy variable was the subject's global assessment of IBS symptoms using a 7-point Likert scale. Patients were asked to answer the daily question "If you consider your IBS symptoms (e.g. abdominal pain/discomfort, distension/bloating, urgency, bowel habit) in general, how have you been affected by these symptoms during the last 24 hours?" Possible answers

ranged from 0 (not at all), 1 (very mild), 2 (mild), 3 (moderate), 4 (strong), 5 (very strong) to 6 (intolerable).

Secondary efficacy variables included “abdominal pain/discomfort”, “distension/bloating”, and “urgency”, recorded on the same 7-point Likert scale. The individual symptom scores were additionally combined into a composite symptom score as the arithmetic mean of three individual symptom scores. Furthermore, the number of bowel movements, feeling of incomplete bowel evacuation and intake of other medications were reported daily in the diary.

At the end of the treatment and again at the end of the study, physicians questioned the patients regarding the global assessment of efficacy, tolerability as well as digestive disorder (“bowel movement satisfaction”). Efficacy was assessed by the following question: “Please consider how you felt during the 4 week treatment regarding your overall well-being, and symptoms of abdominal discomfort/pain and altered bowel habit. Compared to the way you usually felt before taking the study medication, how would you rate your relief of symptoms during the last 4 weeks?” Possible answers were: “completely relieved (1), considerably relieved (2), somewhat relieved (3), unchanged (4) or worse (5)”. Both “completely relieved” and “considerably relieved” were defined as “adequate relief”.

Health related quality of life was assessed by the use of the SF-12 questionnaire prior to the treatment and at the end of the treatment.

Adverse events were recorded throughout the study and the global assessment of tolerability was questioned at physician visit 3 and 4. Laboratory values and vital signs were examined at the screening visit and at the end of the study.

2.5 Statistical methods

Sample size estimation

A reduction of the subject's global assessment (SGA) of at least 20% on the 7-point Likert scale was considered as a relevant treatment effect. Based on published data (17), a difference of 0.6 points in the SGA of IBS symptoms between *B. bifidum* MIMBb75 and placebo on the 7-point Likert scale was anticipated (e.g. 3.0 in the placebo group and 2.4 in the bifidobacteria group). Standard deviation was estimated with 1.0 using the same data. With these assumptions, a Wilcoxon-Mann-Whitney test with a two-sided significance level of α=0.05 and a power of 1- β = 0.8, a sample size of 47 patients per group was required. With an estimated drop-out rate of 15-20% after randomization, 110 randomized patients were planned and 132 patients were recruited to account for possible withdrawals prior to the start of the study.

Statistical analysis

The primary objective of this study was to prove a significant reduction of the SGA of general IBS symptoms at the end of the treatment in the bifidobacteria group vs. placebo. The SGA was calculated for each subject as arithmetic mean at baseline, during the treatment period and during the wash-out phase. To account for possible differences in the baseline values, the change from baseline calculated as mean score during 4 weeks of treatment minus mean score during the run-in phase (week 1-2) was defined as primary target criterion. The non-parametric Van Elteren test stratified by study centers was used for the comparison of treatment arms. P<0.05 was considered statistically significant.

The primary analysis was based on the intent-to-treat population where all successfully randomized patients were included. Missing post-baseline values were imputed by the baseline value for the primary target criterion and these patients were evaluated as non-responders (n=1 during treatment, n=3 during wash-out phase). An additional per protocol analysis was performed for supportive purposes.

Descriptive analyses of secondary target criteria were based on available data. Treatment differences were tested by the use of the non-parametric Wilcoxon test for continuous variables or by Fishers exact test for binary variables. All p-values are two-sided.

Secondary efficacy variables included response based on a 50% rule of symptom relief during the treatment (at least improvement in two out of four weeks within the treatment period and improvement defined as at least one point reduction from baseline).

All statistical analyses were perfomed using SAS version 9.1.3 for windows, SAS Institute Inc., Cary, NC.

3. Results

Subjects

A total of 132 patients were included into the study and 122 patients were successfully randomized to receive either placebo (N=62) or *B. bifidum* MIMBb75 (N=60). All randomized patients were analyzed for intent to treat (N=122). One patient with no post randomization visit was excluded from the analysis of adverse events. A total of 103 patients (49 placebo, 54 *B. bifidum* MIMBb75) were examined as per protocol (Fig. 2).

Baseline Characteristics

In terms of baseline characteristics, there were no significant differences between the groups. 21.3% were classified as diarrhea-predominant IBS (23.3% in the bifidobacteria group, 19.4% in the placebo group), 19.7% as constipation-predominant IBS (15% in the bifidobacteria group, 24.2% in the placebo group) and

58.2% as alternators (61.7% in the bifidobacteria group, 54.8% in the placebo group) with no significant differences between the bifidobacteria and the placebo group. Demographics were well balanced between the treatment groups with about 67% female patients and mean weight of 71 kg corresponding to a BMI of 24. The average age of patients was 41 years in the placebo group and 37 years in the bifidobacteria group (Table 1).

Subject's global assessment (SGA) of IBS symptoms

The primary endpoint was the reduction of the SGA of IBS symptoms on the subject's global assessment diary. *B. bifidum* MIMBb75 significantly improved global IBS symptoms by -0.88 points [95%CI: -1.07; -0.69] (from 2.95 in the run in phase to 2.07 in the treatment phase) compared to only -0.16 points [95%CI: -0.32; 0.00] (from 2.79 in the run in phase to 2.63 in the treatment phase) in the placebo group ($p<0.0001$) using the 7-point Likert scale. The evaluation of the SGA on a weekly basis showed a significant benefit for patients within the bifidobacteria group for every single week starting the second week of treatment till the end of the study (Fig. 3).

Secondary endpoints

Secondary endpoints included changes in IBS symptoms – "pain/discomfort", "distension/ bloating", "urgency", "number of bowel movements" and "feeling of incomplete evacuation" - on a seven-point Likert scale. *B. bifidum* MIMBb75 showed a significant reduction of pain/discomfort by -0.82 points [95%CI: -1.01; -0.63] vs. -0.18 [95%CI: -0.35; -0,01] in the placebo group ($p<0.0001$), and distension/bloating by -0.92 points [95%CI: -1.15; -0.69] vs. -0.21 [95%CI: -0.37; -0.05] in the placebo group ($p<0.0001$) during the treatment. The

reduction persisted during the wash-out phase. Urgency was significantly reduced by -0.67 points [95%CI: -0,86; -0,48] vs. -0.21 [95%CI: -0,35; -0.07] in the placebo group (p=0.0001) during the treatment but not during the wash-out phase. No effects could be detected for frequency of bowel movement and feeling of incomplete bowel evacuation (Fig. 4).

The evaluation of the IBS symptoms pain/discomfort and distension/bloating on a weekly basis showed a significant benefit for patients within the bifidobacteria group compared to placebo for every single week beginning the second week of treatment till the end of the study. A significant difference in urgency between the bifidobacteria and the placebo group was shown between week four and six (Fig. 5 and Fig. 6).

Bowel movement satisfaction (digestive disorder) decreased from 3.89 to 2.44 in the bifidobacteria group vs. 3.69 to 3.47 in the placebo group (p=0.0002) after treatment. The reduction persisted during wash-out phase (2.33 in the bifidobacteria group vs. 3.47 in the placebo group, p<0.0001).

Composite score

A composite score was calculated for the IBS symptoms pain/discomfort, distension/bloating and urgency. During the run in phase, the score was comparable in both groups. The patients within the bifidobacteria group significantly benefited from the consumption of *B. bifidum MIMBb75* vs. placebo. The composite score improved from 2.68 at baseline to 1.88 after treatment in the bifidobacteria group and from 2.56 at baseline to 2.37 after treatment in the placebo group (-0.80 in the bifidobacteria group; -0.20 in the placebo group; p<0.0001). This improvement was

also preserved during the wash-out phase (-0.85 in the bifidobacteria group; -0.31 in the placebo group; p<0,0001).

Treatment responders

Overall responders were defined as patients experiencing an improvement of the average weekly score of at least 1 point on the Likert scale for the primary parameter (SGA of IBS symptoms) in at least two out of the 4 weeks treatment period (50% rule). Abdominal pain responders were defined using the same 50% rule for at least one point average improvement for the assessment of “pain/discomfort”. Overall responder rates were 56.7% in the bifidobacteria group and only 21.0% in the placebo group (p=0.0001). The difference between the treatment arms was only a little bit less pronounced when considering only the symptom “pain/discomfort” where responder rates were calculated to be 48.3% in the bifidobacteria and only 24.2% in the placebo group (p=0.008) (Fig. 7).

Global efficacy at physician site

The overall assessment of efficacy was significantly better in the bifidobacteria group compared to placebo. At the end of treatment 43.3% of the patients in the bifidobacteria group achieved adequate relief compared to only 8.1% in the placebo group (p<0.0001). At the end of the study adequate relief was reported for 46.7% in the bifidobacteria and 11.3% of the patients in the placebo group (p<0.0001; Fig. 8).

Health related quality of life

The evaluation of the SF12 sum scores showed a significant gain in quality of life within the bifidobacteria group. Physical health sum improved from 47.89 at baseline to 51.80 after treatment in the bifidobacteria group and from 47.33 to only 48.85 in

the placebo group. Physical health sum significantly changed by 3.99 in the bifidobacteria group and by only 1.08 in the placebo group compared to baseline (p=0.0185). Mental health sum improved from 45.53 at baseline to 51.11 after treatment in the bifidobacteria group and from 47.01 to only 48.29 in the placebo group. Mental health sum significantly changed by 5.78 in the bifidobacteria group and by only 1.58 in the placebo group compared to baseline (p=0.0083).

Adverse events

Only 36 adverse events were reported with suspected relation to the study product, 13 in the placebo and 23 in the treatment group, but no significant differences could be detected in the side effects profile of *B. bifidum MIMBb75* vs. placebo. Additionally, no severe adverse events have been recorded in either group.

4. Discussion and Conclusion

This randomized, double blind, placebo-controlled study indicates that B. bifidum MIMBb75 has beneficial effects in the treatment of IBS. In this study, B. bifidum MIMBb75 significantly improved global IBS as well as its related symptoms such as pain/discomfort, distension/bloating and digestive disorders compared to placebo. Moreover, B. bifidum MIMBb75 also significantly improved the quality of life. These benefits persisted within the consumption-free wash-out phase. Overall responder rates were predominantly high with 56.7% in the bifidobacteria group compared to only 21.0% in the placebo group (p=0.0001). At the end of the study adequate relief

was reported for 46.7% in the bifidobacteria and only 11.3% of the patients in the placebo group (p<0.0001).

Up to date, several studies have examined the effects of probiotics on IBS and its symptoms (13, 14, 18, 19). However, only a few could show a significant benefit. Additionally, to our knowledge, no probiotic strain could show to significantly alleviate irritable bowel syndrome and simultaneously improve the quality of life. While some studies might have missed to show efficacy due to small sample size and randomization errors, several different probiotic strains did repeatedly show no significant improvement in IBS (20, 21, 22, 23, 24). Recently, Brenner et al. (2009) published a systematic review of randomized controlled trials (RCTs) aimed at the evaluation of the efficacy, safety, and tolerability of probiotics in the treatment of IBS. A total of 16 RCTs were included in the analysis. Of those, exclusively one bifidobacteria strain showed efficacy for improvement of IBS symptoms in two appropriately designed studies (4). Apart from inappropriate study design, in our opinion, these findings could be attributed to the fact that the efficacy of probiotics is strongly strain specific and that only few strains might be able to show efficacy in IBS.

No study to date could prove unambiguously the mode of action of probiotics, which can be clearly linked to the improvement of IBS and its symptoms. Several studies have observed altered intestinal microbiota with a significant lack of bifidobacteria (25, 26) and a dysfunctional intestinal barrier in IBS patients. These studies have been supported by the fact that a normalization of the lactulose breath test, which suggests the presence of small intestinal bacterial overgrowth or an increased number of enteric microorganisms, is highly correlated with a significant reduction of IBS symptoms (27). It has been stated that the imbalance of the microbiotic composition may lead to a different fermentation pattern, especially with increased

hydrogen production resulting in bloating (28, 29). Additionally, gut mucosal barrier disruption has been proven to be associated with bacterial translocation and inflammatory conditions (4,5,6). It seems likely that only specific strains are able to restore an imbalanced intestinal microbiota and gut barrier, however further research is needed to investigate if the positive effects of *B. bifidum* MIMBb75 can be attributed to this mode of action.

There are several limitations of this study. First of all, the study population was not large enough for a sub-group analysis of IBS subtypes. We could therefore not show whether some IBS-subtypes would benefit more from the consumption of B. *bifidum* MIMBb75 than others. In order to prove whether some subgroups might benefit more, a larger study might be useful. The study population has mild to moderate IBS symptoms as evidenced by the indicated SGA-baseline values. A further study should be aimed at the examination whether the patients with severe IBS symptoms will profit in the same way as those with mild to moderate symptoms or whether these patients are more refractory to this treatment.

Additionally, the duration of the study was perhaps short at 4 weeks. It would be of specific value to explore whether patients benefit more from a longer consumption of B. *bifidum* MIMBb75. Finally, the observation that patients still benefit during the consumption-free wash-out phase is of significant value and requires further investigation (for longer follow-up).

In conclusion, the beneficial effects of *B. bifidum* MIMBb75 in improving global IBS as well as its symptoms along with the good side effect profile suggests that this probiotic strain has the potential as an effective alternative to current treatment options.

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Statement of Interests

Author's declaration of personal interest: none.

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Tables

Table 1: Demographic characteristics of the ITT-population

Table 2: Summary of reported adverse events with suspected relationship by system organ class (SOC)

Table 3: Incidence of adverse events at visit 3-4 by preferred term (occurrence of more than 2 %)

Table 2

	Placebo	*Bifidobacterium bifidum* MIMBb75
	N(%) or Mean ± SD	N(%) or Mean ± SD
N=122 (62+60)		
Age	40.98 ± 12.80	36.65 ± 12.42
Female sex	41 (66.1)	41 (68.3)
Height (cm)	169.50 ± 8.75	170.78 ± 9.47
Weight (kg)	70.79 ± 15.54	70.45 ± 16.02
BMI	24.60 ± 5.19	24.02 ± 4.45
IBS Type (N=122 (61+60))		
Diarrhea predominat	12 (19.4)	14 (23.3)
Constipation predominat	15 (24.2)	9 (15.0)
Alternating type	34 (54.8)	37 (61.7)

Table 2

		Group					
		Placebo		Verum		All	
		N	PctN	N	PctN	N	PctN
Visit	SOC						
3	Gastrointestinal disorders	7	53.85	12	52.17	19	52.78
	General disorders and administration site conditions	1	7.69	.	.	1	2.78
	All	8	61.54	12	52.17	20	55.56
4	SOC						
	Gastrointestinal disorders	5	38.46	8	34.78	13	36.11
	General disorders and administration site conditions	.	.	1	4.35	1	2.78
	Investigations	.	.	1	4.35	1	2.78
	Skin and subcutaneous tissue disorders	.	.	1	4.35	1	2.78
	All	5	38.46	11	47.83	16	44.44
All		13	100.00	23	100.00	36	100.00

Table 3

	Treatment N	(%)	Control N	(%)	Total N	(%)	P
Abdominal distension	3	5.00	4	6.56	7	5.79	1.0000
Abdominal pain	2	3.33	2	3.28	4	3.31	1.0000
Abdominal pain upper	3	5.00	1	1.64	4	3.31	0.3645
Constipation	2	3.33	1	1.64	3	2.48	0.6187
Diarrhoea	3	5.00	1	1.64	4	3.31	0.3645

	Treatment N	(%)	Control N	(%)	Total N	(%)	P
Frequent bowel movements	3	5.00	0	0.00	3	2.48	0.1188
Nausea	3	5.00	0	0.00	3	2.48	0.1188

All p values by Fishers exact test.

Figures

Fig. 1. Study schematic.

Fig. 2. Diagram of study flow.

Fig. 3. Comparison of effects of placebo and *B. bifidum* MIMBb75 on global IBS symptoms (by SGA, recorded on a 0-6 scale) on a weekly basis. Significant improvement of global IBS symptoms in the bifidobacteria group vs. placebo.

Fig 4.. Comparison of the reduction of IBS symptoms (*B. bifidum* MIMBb75 vs. placebo) on mean score changes from baseline to treatment phase.

Fig. 5. Comparison of effects of placebo and *B. bifidum* MIMBb75 on pain/discomfort (recorded on a 0-6 Likert scale) on a weekly basis. Significant improvement in the bifidobacteria group vs. placebo group.

Fig. 6. Comparison of effects of placebo and *B. bifidum* MIMBb75 on distension/bloating (recorded on a 0-6 scale) on a weekly basis. Significant improvement in the bifidobacteria group vs. placebo group.

Fig. 7. Overall responders during treatment (ITT).

Fig. 8. Adequate relief after treatment (ITT).

Fig.1

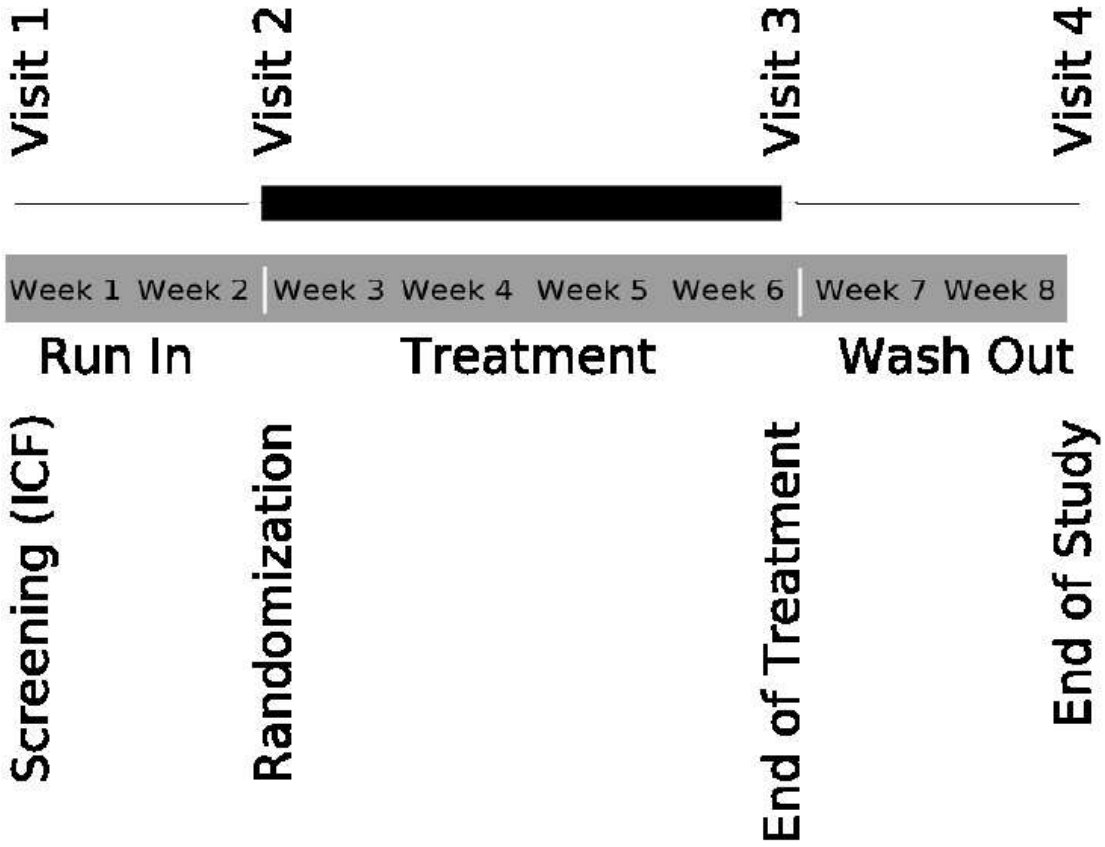


Fig.2.

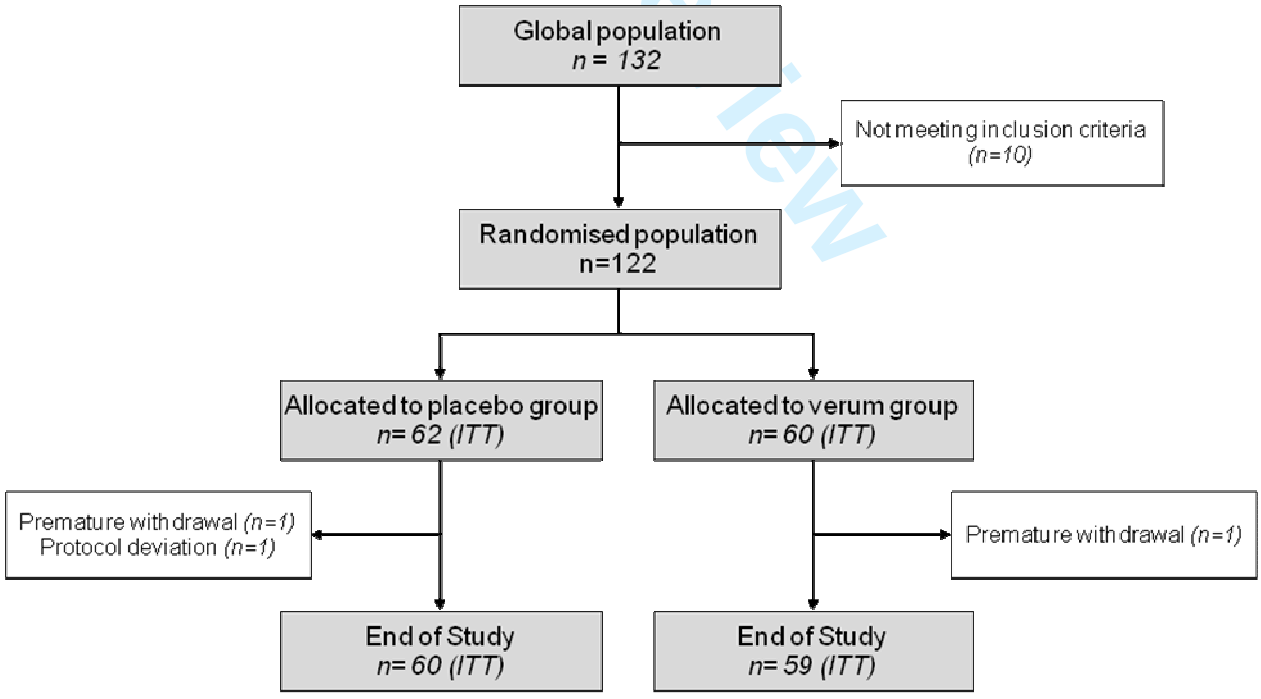


Fig. 3

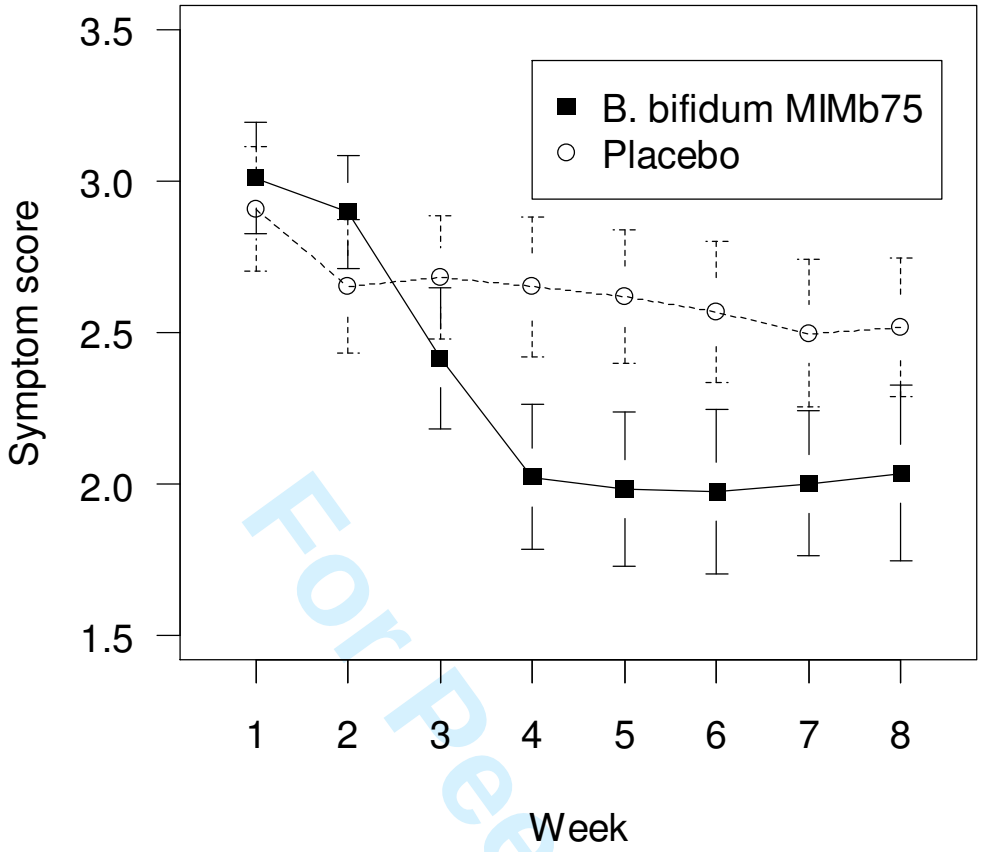


Fig.4

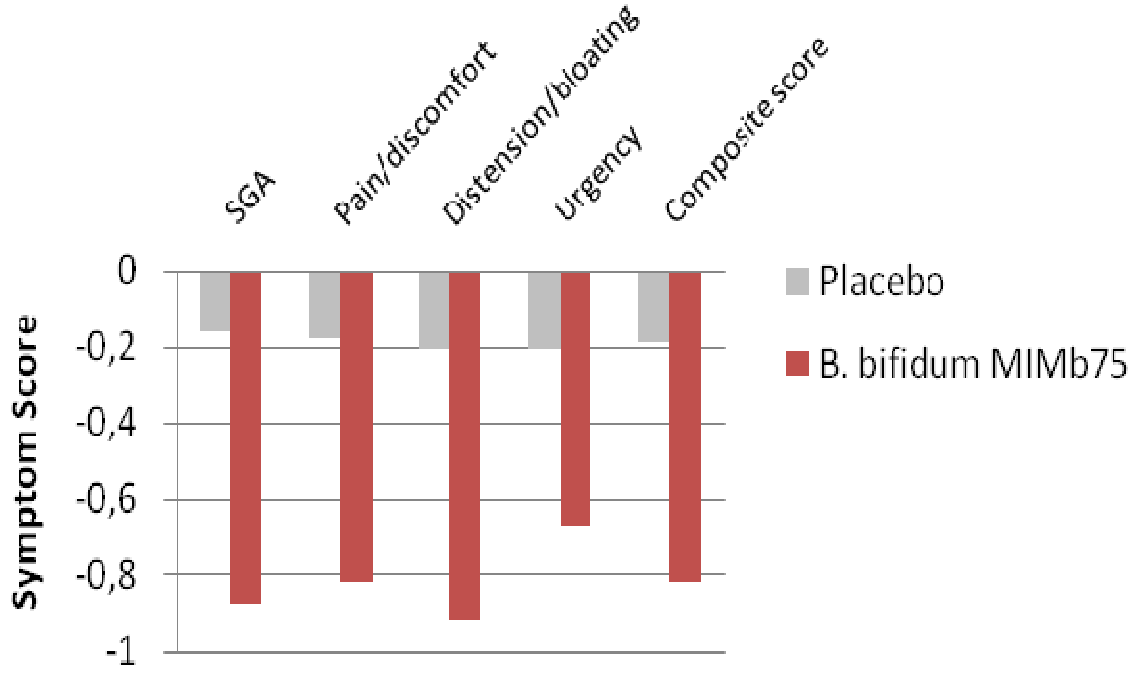


Fig. 5

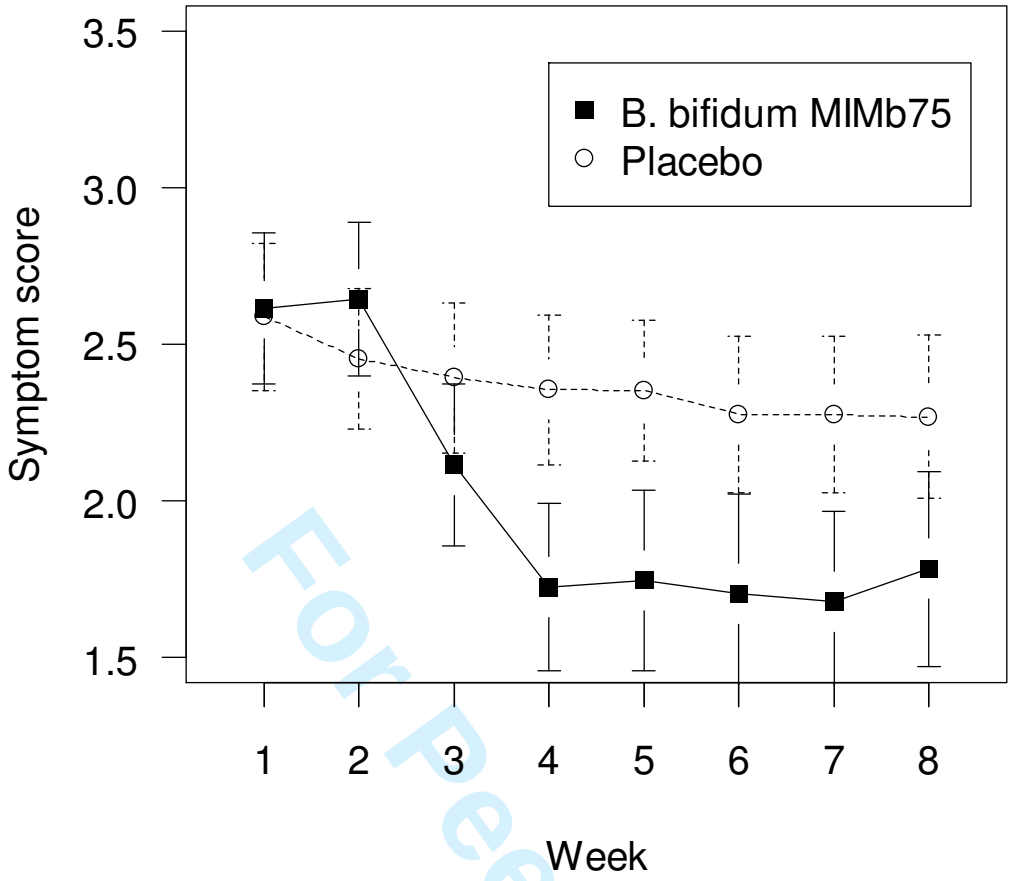


Fig. 6

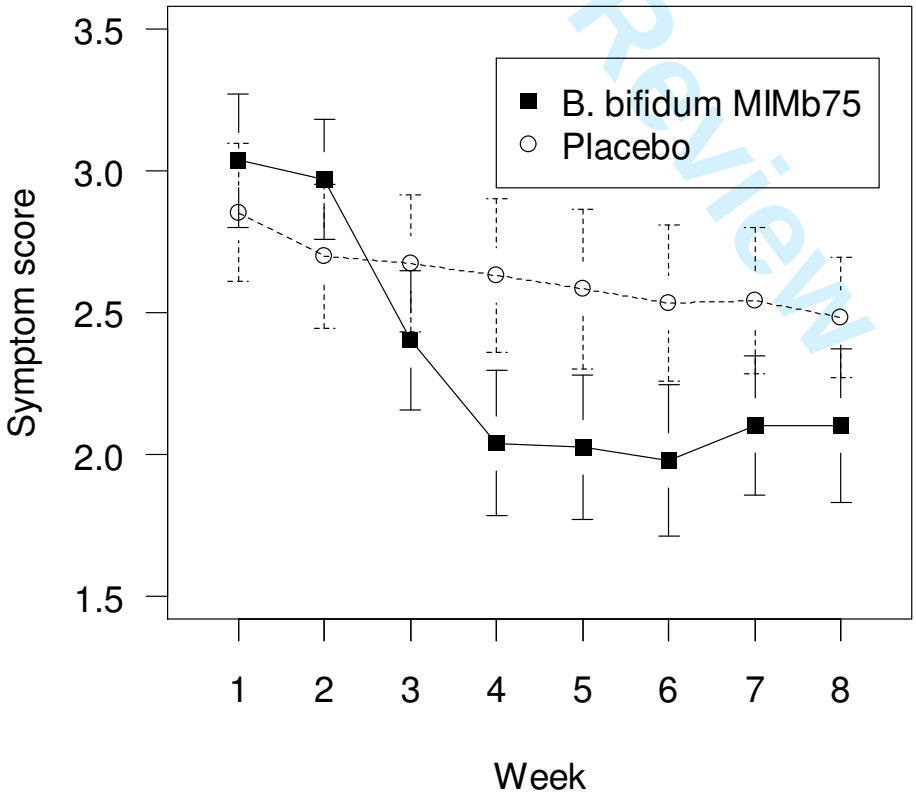


Fig. 7

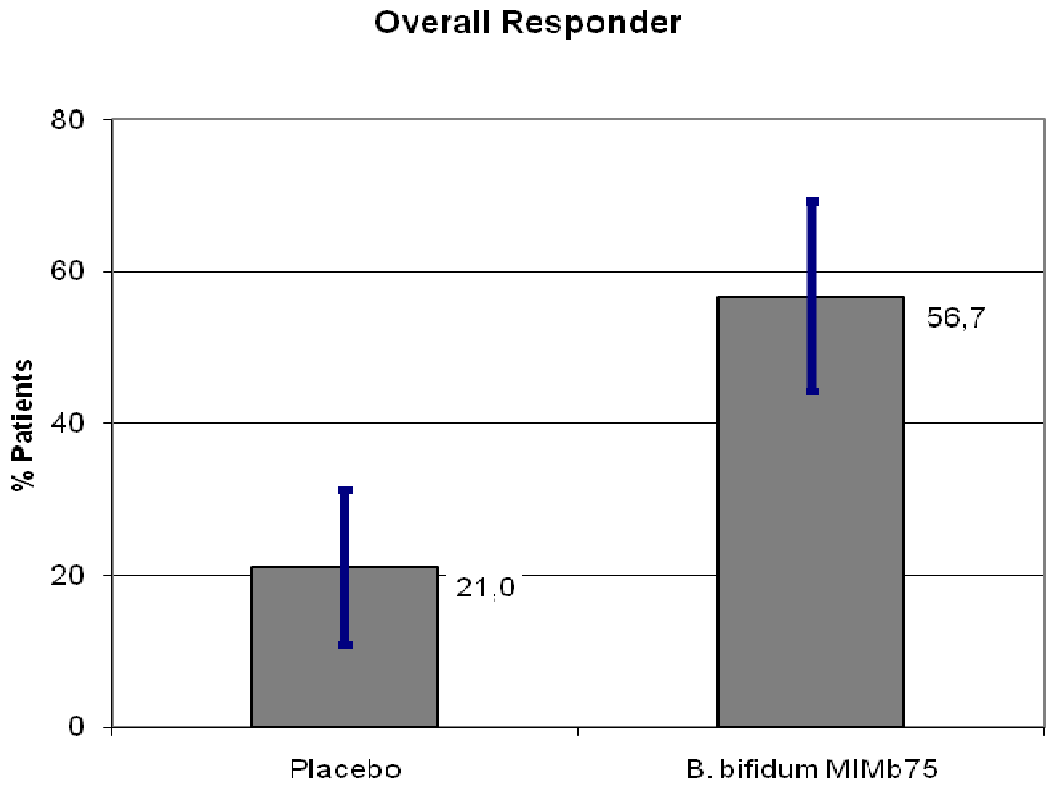
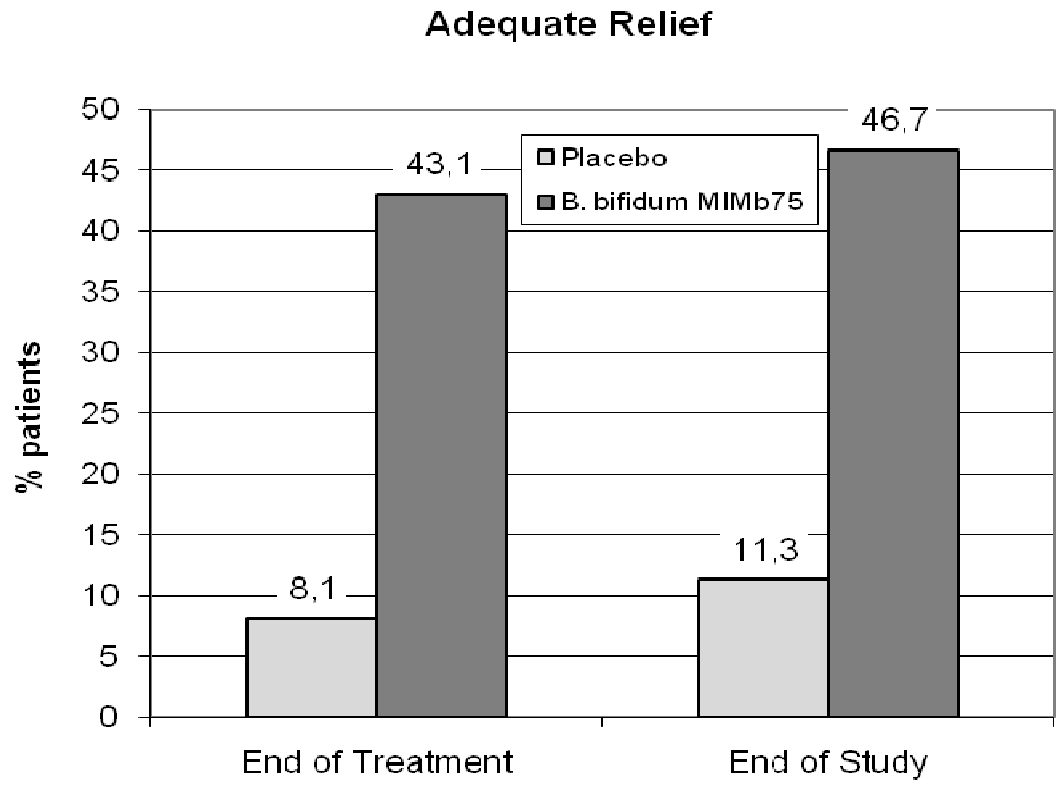
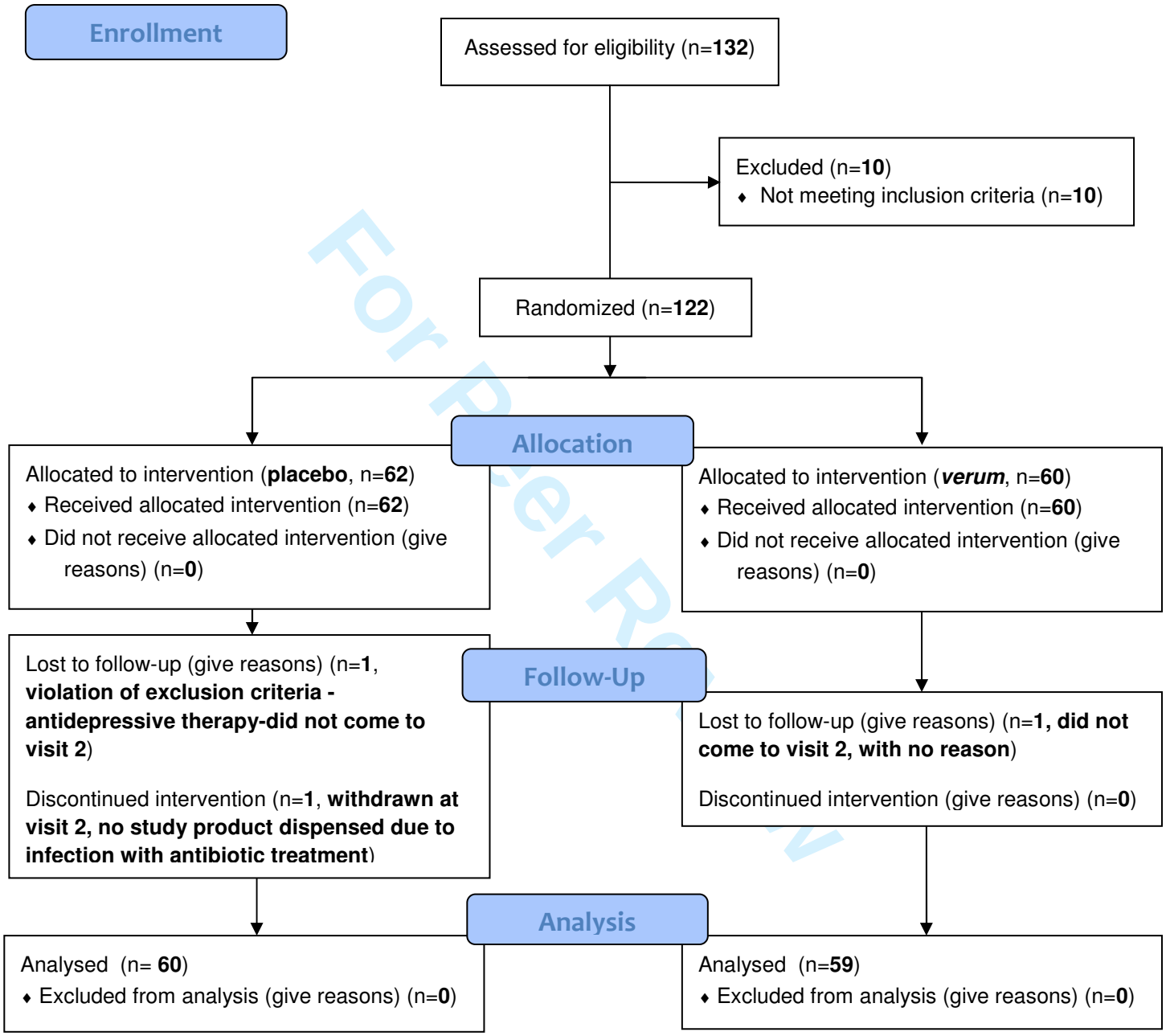

Overall Responder
% Patients
80
60
40
20
0
21,0
56,7
Placebo
B. bifidum MIMb75

Fig. 8


Adequate Relief
% patients
50
45
40
35
30
25
20
15
10
5
0
43,1
46,7
8,1
11,3
Placebo
B. bifidum MIMb75
End of Treatment
End of Study

CONSORT 2010 Flow Diagram



CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	3
	2b	Specific objectives or hypotheses	3
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	4, 5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	/
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	4-7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	4-7
	6b	Any changes to trial outcomes after the trial commenced, with reasons	/
Sample size	7a	How sample size was determined	8
	7b	When applicable, explanation of any interim analyses and stopping guidelines	/
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	/
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	5

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	/
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7, 8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8, 9
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9
	13b	For each group, losses and exclusions after randomisation, together with reasons	CONSORT Flow Diagram
Recruitment	14a	Dates defining the periods of recruitment and follow-up	4, 5
	14b	Why the trial ended or was stopped	/
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	20
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	/
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	/
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	Fig. 1-8
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	/
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	13
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	15
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	13, 14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-15
Other information			
Registration	23	Registration number and name of trial registry	/
Protocol	24	Where the full trial protocol can be accessed, if available	/
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	18

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.