Randomised double blind placebo controlled clinical trial to evaluate the effect of a mixture of probiotic strains on symptom severity and the use of corticosteroids in children and adolescents with atopic dermatitis

Running head: The Gut-Skin Axis: Probiotics, Microbiome and Atopic Dermatitis

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What is already known about this topic?

- The gut-skin axis concept and the relationship between atopic dermatitis and the gut Microbiota is an increasingly documented fact and many researchers are investigating the effect of oral administered of probiotic strains on this microbiota and on the evolution of this skin disease.

What does this study add?

- This confirmatory study builds on the existing dataset for this combination probiotic strains in the management of atopic dermatitis in children and adolescent. Results confirm the beneficial effect of the probiotic on symptoms of atopic dermatitis. Also, between 6-12 weeks, the probiotic group shows a reduction in the dose of topical corticosteroids used, compared to placebo.

ABSTRACT

Background: Intestinal microbiota is altered in patients with Atopic Dermatitis (AD) when compared to that of the healthy population. Some interventions with specific probiotic preparations already demonstrate a change in the composition of this microbiota accompanied by an improvement of the disease.

Objectives: This research work, is designed to evaluate the clinical efficacy of the probiotic and to measure the effect of the intervention on the total dose of corticosteroids administered to subjects.

Methods: A double-blind, randomized, placebo-controlled clinical trial including AD participants, aged 4 to 17 years, to evaluate the clinical effect of a probiotic mixture of *Bifidobacterium lactis*, *Bifidobacterium longum* and *Lactobacillus casei* in a total daily consumption of $1x10^9$. The analyzed clinical variables are the SCORAD (Scoring of Atopic Dermatitis) and IGA (Investigator Global Assessment) indices, the effect on the consumption of topical corticosteroids and the assessment of safety.

Results: SCORAD index score at 12 weeks shows a statistically significant difference of -5.43 (-10.65 to -0.21) between the probiotic (SCORAD 13.52) and placebo groups (SCORAD 18.96); p=0.04. Comparison between groups shows statistically significant

difference in the number of patients with IGA score improvement over the 12-week intervention: 29 (90.5%) patients in the probiotic group and 17 (56.7%) patients in the placebo group (p<0.002). A comparison between groups of the proportions of days using corticosteroids and the total grams of corticosteroids between baseline and end of study shows no significant difference, but between weeks 6-12 there is a statistically significant reduction in the probiotic group when compared with the placebo group in both variables. The numbers of adverse events are similar in both groups of treatment.

Conclusions: T The probiotic used in this clinical trial demonstrates efficacy on the change of the activity index of AD compared to placebo. The total number of days and total amount of topical corticosteroids required by the subjects in the probiotic group showed a significant reduction compared to placebo between 6-12 weeks.

BACKGROUND

Atopic dermatitis (AD) affects 8% of the population worldwide, with a majority being diagnosed during childhood or adolescence.[1] Mild to moderate cases are usually treated with emollients, topical steroids, and phototherapy. In severe cases, oral corticosteroids and other systemic immunosuppressants, including cyclosporine, methotrexate sodium or azathioprine sodium may be required.[2] The use of monoclonal antibodies and janus kinase inhibitors has shown promising results in terms of both efficacy and safety.[3]

Environmental factors, immunological system and genetics are all thought to contribute to the complex pathophysiological mechanism of AD.[4] Over recent years the relationship between the human microbiome and AD has been hypothesized, arguing the implication of gut microbiota on the pathophysiology of AD.[5,6] Thanks to the High Throughput Sequencing technology, we are developing a greater understanding of the microorganisms that colonize our intestines as well as many of its functions. One of these functions is its contribution to the degradation of complex indigestible polysaccharides and its essential role in the production of certain nutrients. Furthermore, short chain fatty acids (SCFAs) resulting from the fermentation of dietary fibre by bacteria in the gastrointestinal tract play a protective role against bacterial translocation from the intestinal lumen. This process has been shown to occur in some inflammatory skin diseases, for example psoriasis where intestinal bacterial DNA has been found in blood samples during symptom flare ups.[7,8] Those patients who have an intestinal microbiome rich in SCFA-producing bacteria have a lower tendency to suffer bacterial translocation phenomena.[9] This

phenomenon may be partly responsible for the interconnection between the intestinal and skin microbiota. Ultimately, this activation would provoke an immune and metabolic response of the skin, which would affect the microbial composition of this organ itself.[10]

Previous studies show how the intestinal microbiota is altered in patients with AD when compared to that of the healthy population.[11] Some interventions with specific probiotic preparations already studied the effect on the gut microbiota, demonstrating a change in the composition of this microbiota accompanied by an improvement of the disease.[12-23]

The probiotic preparation studied in this clinical trial has already demonstrated efficacy on the change of the activity index of AD and on the total number of days that the subjects in the study required steroid treatment for the disease.[24] This new research work, using the same blend and same dose, is designed to evaluate not only the clinical efficacy of the probiotic (and confirm previous results), but also to measure the effect on the total dose of corticosteroids administered to subjects and also the effect on the time free of new outbreaks after finishing the treatment with the product under research.

METHOD

Study Design

A double-blind, randomized, placebo-controlled clinical trial. The study received approval from the Ethics Committee for Clinical Research of the University Hospital Ramón y Cajal (Madrid, Spain), and was registered in the American Registry of Clinical Trials (NCT03822624).

Study population

Participants in the study were aged 4 to 17 years, both male and female, with diagnosis of AD according to Hanifin-Rajka criteria and SCORAD (Scoring of Atopic Dermatitis) index of 20–40.[25] Patients were excluded from the study if they met any of the following conditions: Pregnancy, breast feeding, women of childbearing age who do not commit to using an effective method of contraception, use of phototherapy for the AD in the previous 2 months, systemic corticosteroids, cytotoxic or immunosuppressive therapy in the previous 2 months or having an anticipated need during the first 12 weeks of the study, probiotics intake in the previous 2 months, systemic antibiotics treatment in the previous four days, fever (temperature > 37.5°C, axillary or equivalent), diseases related to

immunodeficiency processes or cancer, other skin diseases that can make AD assessment difficult or which require the continuous use of topical corticosteroids, patients in whom any of the study products is contraindicated, patients who have participated in research studies with medicinal products during the previous 3 months, patients with gluten or lactose intolerance or inflammatory bowel disease (Crohn's disease or ulcerative colitis). Before being included in the study patients and parents or legal guardian agreed to participate in the study by signing a written informed consent to participate in the trial.

Recruitment, Randomization and Masking of Participants

Participants were recruited among five different public University Hospital dermatological departments and one private outpatient clinic in Spain by dermatologists with expertise in paediatric dermatology. A physician assigned each patient a study participant number from a stratified randomised list drawn up considering gender, patient age and disease onset age variables. In each stratum, the numbers in the list were randomised to each of the two intervention groups, to minimise the imbalance between groups.

Intervention

The treatment evaluated in this study was the oral administration of one capsule daily of a probiotic consortia containing *Bifidobacterium animalis* subsp. *lactis* CECT 8145, *Bifidobacterium longum* CECT 7347 and *Lactobacillus casei* CECT 9104 at a concentration of 1x10^9 cfu (colony-forming unit) per capsule with maltodextrin as anti-caking agent, for a period of 12 weeks. This mixture has been selected due to the promising effective results when tested in previous preclinical studies[26-29] and in a pilot clinical trial in patients with moderate AD.[24] The control product was the placebo, of a similar appearance to the probiotic preparation, containing maltodextrin powder. The product was delivered by the pharmacy service of the centre involved or the hospital dermatological department attending patients.

Other unblinded medication allowed during the study and similar in both groups were: Topical corticosteroids methylprednisolone aceponate or diflucortolone valerate 0.05%, applied to the affected area during flare-ups once a day in a thin layer and gently rubbed in, adjuvant emollient treatment recommended in skin that becomes very dry during the study, systemic corticosteroids, oral antihistamines, topical antibiotics. All medications taken by the patient during the study was recorded, specifying dose, administration route and treatment duration.

Outcome measures

The primary outcome measured was the difference in SCORAD index between groups at the end of the study period. Secondary variables were: The number of days that each patient requires topical corticosteroids administration over the 12 weeks of the study. The total dose of topical corticosteroids used during the intervention. The number of subjects with improvement in the IGA (Investigator Global Assessment) scale score of 1 point or greater from baseline. The number of adverse events by group.

Study visits and procedures

The study was structured in 6 visits, three of those face-to-face: Visit 1 when subjects were included in the study; visit 4 in week 6 since the beginning of the oral intake of the products and visit 6 when the study product intake ends at week 12. Other three visits were telephone evaluation visits at weeks 2, 4 and 9 from inclusion of the patients in the study (Supplementary material-I).

Assessment of safety

A table was drawn up on the frequency of each type of adverse event as well as the proportions of patients with the events, compared between the two groups. The investigator made every possible effort to explain each adverse event and to evaluate its possible relationship to the study product.

Sample size

Data from previous studies with a similar diagnosis and moderate severity have shown that after a 12-week follow-up the mean SCORAD score fell an average of 8 and 27 points in the control and intervention groups respectively, and the standard deviations of those changes were about 6 and 11 points in each group after a 12-week follow-up. It was therefore estimated that with 35 patients per group we could detect a clinically relevant difference between the two groups of 5.3 points in the mean change in the SCORAD index from baseline, with a power of 80%, a Type I error of 0.05 and estimated losses of 10% of subjects.

Data analysis and evaluation

The continuous variables were summarised by means and standard deviation and categorical variables with absolute and relative frequencies. Primary analysis was conducted on an intention-to-treat basis, including each patient in the analysis within the

group assigned to that patient after randomization. For the analysis of the results, all available data are used up to the last visit made by each randomized patient. The statistical analysis was done using the computational R 3.3.2 package.

A mixed linear regression model in which the individual is kept as the random factor was used to compare the difference of the change from baseline in the SCORAD, between the two study groups in the different visits. The normality was analyzed using the Kolmogorov-Smirnov test. P-values were obtained using a wald-test of the linear combinations of interest. Values at visits 4 and 6 were obtained assuming the SCORAD at baseline was equal to its mean (i.e., the SCORAD at baseline centred at its mean was equal to zero). Two arms Fisher's test was used to evaluate IGA variable comparing, between treatment groups, the proportion of patients that improved IGA score in at least one point at each face-to face visit. Pearson's chi-squared for the grouped values was used to compare the proportion of days using corticosteroids per the number of days in the study by group during an interval between visits, and the non-parametric Mann-Whitney-Wilcoxon test after verifying the homoscedasticity of the data was used to compare the intake of corticosteroids by group. Finally, the Chi-squared tests of homogeneity comparing the numbers of adverse events by group and a Mann-Whitney-Wilcoxon test comparing of treatment adherence by group was used.

RESULTS

From October 2018 to February 2021, children with a diagnosis of AD were enrolled in the study. Patients' data included in the study comparing the two groups of variables at baseline visit appears in descriptive Table 1. Information related with assessed patients for eligibility, randomized and finally analyzed is included in the consort diagram. Reasons of exclusion in any step of the study and number of patients with lost to follow up in each one group of treatment are also included (Figure 1).

Assessment of efficacy: SCORAD index

Fitted values and differences of the SCORAD index between groups at each visit appear in Figure 2. Trajectories of SCORAD index diverge at the end of the 12 weeks intervention and shows a statistical significance difference of -5.43 (-10.65 to -0.21); p=0.0416, among participants treated with the placebo (Baseline SCORAD 33.51 and final follow up SCORAD 18.96) when compared with data in patients in the probiotic group

(baseline SCORAD 33.68 and final follow up SCORAD 13.52). Difference between groups in the SCORAD subcomponents at each visit are included in Table 2.

Assessment of efficacy: Investigator Global Assessment

Comparison between groups shows statistically significant difference in the number of patients with IGA score improvement of 1 point or greater from baseline over the12-week intervention: 29 (90.5%) patients in the probiotic group and 17 (56.7%) patients in the placebo group ($p<0.002$). On the other hand, analysis of differences between both groups of intervention at each face-to-face visit during the study are included in Figure 3.

Days of topical corticosteroids use

A comparison between groups of the proportions of days using corticosteroids per the number of days in the study was performed. Between baseline and end of study there is no significant difference, however a post-hoc analysis of the data between weeks 6-12 shows a statistically significant reduction in the probiotic group compared with the placebo group (Table 3).

Total use of topical corticosteroids

A comparison of the total amount of corticosteroids administered by group in the specified interval is shown in Table 4. There is no significant difference in the amount of corticosteroid administered between baseline and end of the study. However in a post-hoc analysis of the data from between weeks 6-12 shows a statistically significant difference in corticosteroid use between the probiotic and placebo groups.

Assessment of safety

The numbers of adverse events between groups of intervention are included in Table 5. There is no significant difference between both groups under treatment.

Adherence to treatment

A comparison of treatment adherence by group, at visits 4 and 6, was performed. No differences were found between probiotic vs placebo groups, with adherence higher than 95% in both groups: 96.6% vs 95.5% in week 6 of intervention and 95.2% vs 97.6% at the end of the intervention.

DISCUSSION

In recent years, several studies have explored the effectiveness of specific probiotic strains, not only in the treatment[12-23] but also in the prevention of AD.[30-33] Current evidence of all these published works suggests that certain probiotic strains could be an option as coadjuvant treatment to improve moderate and severe AD recovery rates in children and adults.[34] Despite the high number of studies and patients included in all these works, there is no strong clinical experience to date among dermatologists and pediatricians in most parts of the world supporting clinical effectiveness of oral probiotics administered to patients with AD. This is in part due to the lack of repeated clinical trials with the same probiotic demonstrating strain-specific effectiveness.[35-39] This fact makes it difficult for clinicians to decide on the choice of a specific probiotic preparation.

The clinical trial reported herein explores the role of a probiotic blend administered to patients with moderate AD. This blend was evaluated in a previous clinical study, the results of which were published in January 2018.[24] The aim of this clinical study is twofold, on the one hand to confirm the previously published findings regarding the efficacy of the product.[24] On the other hand, to demonstrate whether the use of topical corticosteroids is lower in patients treated with the probiotic. To our knowledge, this last point has not been previously analyzed meanwhile most of the previous studies focused on the clinical response, the effect on the immune system or the effect on the intestinal microbiota.

Our results confirm that administration of this specific probiotic blend is an effective coadjuvant treatment for reducing the severity of AD with significant reduction in SCORAD and IGA indices. The total consumption of corticosteroids does not show statistically significant difference during the 12-week treatment, but on sub-group analysis, (established as secondary variable in the approved study protocol), during the second half of the intervention the use of topical corticosteroids was lower in the probiotic group.

The first study protocol using the same probiotic blend was more restrictive with diet limitations to participants that the protocol of this new study recruiting patients in a normal clinical practice situation. Hence, patients under different type of diets participated and allowed researchers to evaluate if this probiotic blend worked, regardless of the patient's diet. Results confirm that administration of this specific probiotic is effective in reducing SCORAD and IGA indices, with statistical significance differences at the end of follow up. These findings are like those previously described with this blend at equal daily dose and corroborates the effectiveness of product. Although differences between both

groups have not been as great as those observed in the first mentioned study, they are similar or even greater than those described in other previous studies using other probiotic strains.[40-50]

Most of the patients were included in the study during the COVID-19 pandemic. It is known that COVID-19 increases the number of patients with low adherence to treatment in some different clinical trials, and that it causes a worse evolution of some concomitant diseases, including among others, AD.[51-57] In fact, an unexpected high percentage of cases without compliance in the protocol study, reaching 20%, appears among our patients.

The total amount of topical corticosteroids use during intervention was lower in the group of patients receiving the probiotic blend when compared to patients included in the placebo group. Data shows how the total oral administration of corticosteroids is similar in both trial arms during the first half of the study, but in the last 6 weeks of the intervention, difference between both groups reach the statistical significance difference. These results are consistent with other data collected in the first clinical study, which showed how difference between groups in the variable “days of use of steroids” started to appear from the middle of the intervention, and the reason is probably due to the delay in the effect of the treatment on the structural changes of the microbiome. Many previously published studies evaluating the effect of probiotics on the gut microbiota, have documented how there is a period of induction necessary for the intestinal microbiota to be modified.[58-60] In the case of AD this time sems to be close to six weeks, as was suggested in the first clinical study done with this probiotic preparation and in the one here communicated.[24]

Considering that diet has not been a criterion for the inclusion of patients and the multicenter design of the trial, an important point of this study is the applicability of these results to patients consuming different types of diet and from different geographic areas in Spain. This data resolves one of the limitations raised by the first study in which only patients with a Mediterranean diet were included.

Some limitations of the study should be considered, including the applicability of our results to patients extended to other population groups such as newborns or adults, still not explored with these specific probiotic strains blend. Another limitation is the high number of patients not receiving allocated intervention after been randomized, due to the problems in the development of clinical trials caused by the COVID-19 pandemic. Other limitation is that it was not possible to collect the cases of recurrence during the three months after the intervention finished, (as planned in the initial protocol), due restrictions to

care patients during the COVID-19 pandemic. In any case, it is to be expected that the outbreaks will continue to occur when patients stop using the probiotic blend, and that new rounds of probiotic treatment or a longer initial treatment can keep patients disease-free for longer.

As conclusion, the results of our study indicate a positive effect of the probiotic in reducing the SCORAD index, the IGA index, and lowering the use of topical corticosteroids in sub-group analysis. This evidence supports the efficacy of administering this probiotic blend to patients with moderate AD and while the sample size in the clinical trial is relatively modest, viewed in conjunction with the existing trial data for this same probiotic, clinicians and patients may consider this probiotic blend as a novel adjunct to conventional topical treatments for AD. Further confirmatory clinical trials in other age groups and other geographical areas are needed to better understand how this microbiome intervention works.

REFERENCES

1 Garg N, Silverberg JI. Epidemiology of childhood atopic dermatitis. *Clin Dermatol* 2015; **33**:281–8.

2 Sedeh FB, Henning MAS, Jemec GBE, Ibler KS. Comparative Efficacy and Safety of Monoclonal Antibodies and Janus Kinase Inhibitors in Moderate-to-severe Atopic Dermatitis: A Systematic Review and Meta-analysis. *Acta Derm Venereol* 2022; **24**:adv00764.

3 Wollenberg A, Kinberger M, Arents B et al. European guideline (EuroGuiDerm) on atopic eczema: part I - systemic therapy. *J Eur Acad Dermatol Venereol* 2022; **36**:1409-1431.

4 D'Auria E, Banderali G, Barberi S *et al*. Atopic dermatitis: recent insight on pathogenesis and novel therapeutic target. *Asian Pac J Allergy Immunol* 2016; **34**:98–108.

5 Lu CY, Ni YH. Gut microbiota and the development of pediatric diseases. *J Gastroenterol* 2015; **50**:720–26.

6 Song H, Yoo Y, Hwang J *et al*. Faecalibacterium prausnitzii subspecies-level dysbiosis in the human gut microbiome underlying atopic dermatitis. *J Allergy Clin Immunol* 2016; **137**:852–60.

7 Vinolo MA, Rodrigues HG, Nachbar RT, Curi R. Regulation of inflammation by short chain fatty acids. *Nutrients* 2011; **3**:858–76.

8 Ramírez-Boscá A, Navarro-López V, Martínez-Andrés A *et al*. Identification of bacterial DNA in peripheral blood from patients with active psoriasis. *JAMA Dermatol* 2015; **151**:670–1.

9 Plöger S, Stumpff F, Penner GB *et al*. Microbial butyrate and its role for barrier function in the gastrointestinal tract. *Ann N Y Acad Sci* 2012; **1258**:52–9.

10 Sümegi A, Szegedi A, Gál M *et al*. Analysis of components of the CD14/TLR system on leukocytes of patients with atopic dermatitis. *Int Arch Allergy Immunol* 2007; **143**:177–84.

11 Climent E, Martinez-Blanch JF, Llobregat L *et al*. Changes in Gut Microbiota Correlates with Response to Treatment with Probiotics in Patients with Atopic Dermatitis. A Post Hoc Analysis of a Clinical Trial. *Microorganisms* 2021; **9**:854.

12 Reddel S, Del Chierico F, Quagliariello A *et al*. Gut microbiota profile in children affected by atopic dermatitis and evaluation of intestinal persistence of a probiotic mixture. *Sci Rep* 2019; **9**:4996.

13 Cukrowska B, Ceregra A, Maciorkowska E *et al*. The Effectiveness of Probiotic Lactobacillus rhamnosus and Lactobacillus casei Strains in Children with Atopic Dermatitis and Cow's Milk Protein Allergy: A Multicenter, Randomized, Double Blind, Placebo Controlled Study. *Nutrients* 2021; **13**:1169.

14 Tan-Lim CSC, Esteban-Ipac NAR, Mantaring JBV 3rd *et al*. Comparative effectiveness of probiotic strains for the treatment of pediatric atopic dermatitis: A systematic review and network meta-analysis. *Pediatr Allergy Immunol* 2021; **32**:124–36.

15 Li L, Han Z, Niu X *et al*. Probiotic Supplementation for Prevention of Atopic Dermatitis in Infants and Children: A Systematic Review and Meta-analysis. *Am J Clin Dermatol* 2019; **20**:367–77.

16 Yin DG, He Z, Duan XY *et al*. Effect of probiotic supplementation during pregnancy and infancy in preventing atopic dermatitis in children: a Meta analysis. *Zhongguo Dang Dai Er Ke Za Zhi* 2019; **21**:82–8.

17 Michelotti A, Cestone E, De Ponti I *et al*. Efficacy of a probiotic supplement in patients with atopic dermatitis: a randomized, double-blind, placebo-controlled clinical trial. *Eur J Dermatol* 2021; **31**:225–32.

18 Kim SO, Ah YM, Yu YM *et al*. Effects of probiotics for the treatment of atopic dermatitis: a meta-analysis of randomized controlled trials. *Ann Allergy Asthma Immunol* 2014; **113**:217–26.

19 Gerasimov SV, Vasjuta VV, Myhovych OO, Bondarchuk LI. Probiotic supplement reduces atopic dermatitis in preschool children: a randomized, double blind, placebo controlled, clinical trial. *Am J Clin Dermatol* 2010; **11**:351–61.

20 Farid R, Ahanchian H, Jabbari F, Moghiman T. Effect of a new symbiotic mixture on atopic dermatitis in children: a randomized-controlled trial. *Iran J Pediatr* 2011; **21**:225–30.

21 van der Aa LB, Heymans HS, van Aalderen WM *et al*. Effect of a new synbiotic mixture on atopic dermatitis in infants: a randomized-controlled trial. *Clin Exp Allergy* 2010; **40**:795–804.

22 Boyle RJ, Bath-Hextall FJ, Leonardi-Bee J *et al*. Probiotics for treating eczema. *Cochrane Database Syst Rev* 2008; **4**:CD006135.

23 Lee J, Seto D, Bielory L. Meta-analysis of clinical trials of probiotics for prevention and treatment of pediatric atopic dermatitis. *J Allergy Clin Immunol* 2008; **121**:116–21.e11.

24 Navarro-López V, Ramírez-Boscá A, Ramón-Vidal D *et al*. Effect of Oral Administration of a Mixture of Probiotic Strains on SCORAD Index and Use of Topical Steroids in Young Patients With Moderate Atopic Dermatitis: A Randomized Clinical Trial. *JAMA Dermatol* 2018; **154**:37–43.

25 Eichenfield LF, Tom WL, Chamlin SL *et al*. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol* 2014; **70**:338–51.

26 Ji H, Li XK. Oxidative stress in atopic dermatitis. *Oxid Med Cell Longev* 2016; **2016**:2721469.

27 Martorell P, Llopis S, González N *et al*. Probiotic strain Bifidobacterium animalis subsp lactis CECT 8145 reduces fat content and modulates lipid metabolism and antioxidant response in Caenorhabditis elegans. *J Agric Food Chem* 2016; **64**:3462–72.

28 Medina M, De Palma G, Ribes-Koninckx C *et al*. Bifidobacterium strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. *J Inflamm (Lond)* 2008; **5**:19.

29 Olivares M, Castillejo G, Varea V, Sanz Y. Double-blind, randomised, placebo-controlled intervention trial to evaluate the effects of Bifidobacterium longum CECT 7347 in children with newly diagnosed coeliac disease. *Br J Nutr* 2014; **112**:30–40.

30 Rø ADB, Simpson MR, Rø TB *et al*. Reduced Th22 cell proportion and prevention of atopic dermatitis in infants following maternal probiotic supplementation. *Clin Exp Allergy* 2017; **47**:1014–21.

31 Simpson MR, Rø AD, Grimstad Ø *et al*. Atopic dermatitis prevention in children following maternal probiotic supplementation does not appear to be mediated by breast milk TSLP or TGF-β. *Clin Transl Allergy* 2016; **6**:27.

32 Chen L, Ni Y, Wu X, Chen G. Probiotics for the prevention of atopic dermatitis in infants from different geographic regions: a systematic review and Meta-analysis. *J Dermatolog Treat* 2022. https://doi.org/10.1080/09546634.2022.2091101.

33 Jiang W, Ni B, Liu Z *et al*. The Role of Probiotics in the Prevention and Treatment of Atopic Dermatitis in Children: An Updated Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Paediatr Drugs* 2020; **22**:535–49.

34 Forster CS, Hsieh MH, Cabana MD. Perspectives from the Society for Pediatric Research: Probiotic use in urinary tract infections, atopic dermatitis, and antibiotic-associated diarrhea: an overview. *Pediatr Res* 2021; **90**:315–27.

35 Wickens K, Black P, Stanley TV *et al*. A protective effect of Lactobacillus rhamnosus HN001 against eczema in the first 2 years of life persists to age 4 years. *Clin Exp Allergy* 2012; **42**:1071–9.

36 Chapman CM, Gibson GR, Rowland I. Health benefits of probiotics: are mixtures more effective than single strains? *Eur J Nutr* 2011; **50**:1–17.

37 Matsumoto M, Aranami A, Ishige A *et al*. LKM512 yogurt consumption improves the intestinal environment and induces the T-helper type 1 cytokine in adult patients with intractable atopic dermatitis. *Clin Exp Allergy* 2007; **37**:358–70.

38 Park CW, Youn M, Jung YM *et al*. New functional probiotic Lactobacillus sakei probio 65 alleviates atopic symptoms in the mouse. *J Med Food* 2008; **11**:405–12.

39 Woo SI, Kim JY, Lee YJ *et al*. Effect of Lactobacillus sakei supplementation in children with atopic eczema-dermatitis syndrome. *Ann Allergy Asthma Immunol* 2010; **104**:343–8.

40 Cukrowska B, Ceregra A, Maciorkowska E *et al*. The Effectiveness of Probiotic Lactobacillus rhamnosus and Lactobacillus casei Strains in Children with Atopic Dermatitis and Cow's Milk Protein Allergy: A Multicenter, Randomized, Double Blind, Placebo Controlled Study. *Nutrients* 2021; **13**:1169.

41 Axt-Gadermann M, Chudomirova K, Noll M. Probiotische Teilbäder bei atopischer Dermatitis [Probiotic baths for atopic dermatitis]. *Hautarzt* 2021; **72**:549–56.

42 Nocerino R, Bedogni G, Carucci L *et al*. The Impact of Formula Choice for the Management of Pediatric Cow's Milk Allergy on the Occurrence of Other Allergic Manifestations: The Atopic March Cohort Study. *J Pediatr* 2021; **232**:183–91.e3.

43 D'Auria E, Panelli S, Lunardon L *et al*. Rice flour fermented with Lactobacillus paracasei CBA L74 in the treatment of atopic dermatitis in infants: A randomized, double- blind, placebo- controlled trial. *Pharmacol Res* 2021; **163**:105284.

44 Rather IA, Kim BC, Lew LC *et al*. Oral Administration of Live and Dead Cells of Lactobacillus sakei proBio65 Alleviated Atopic Dermatitis in Children and Adolescents: a Randomized, Double-Blind, and Placebo-Controlled Study. *Probiotics Antimicrob Proteins* 2021; **13**:315–26.

45 Zareie E, Mansouri P, Hosseini H *et al*. Effect of oral administration of Triphala, a polyphenol-rich prebiotic, on scalp sebum in patients with scalp seborrhea a randomized clinical trial. *J Dermatolog Treat* 2022; **33**:1011–6.

46 Aldaghi M, Tehrani H, Karrabi M *et al*. The effect of multistrain synbiotic and vitamin D3 supplements on the severity of atopic dermatitis among infants under 1 year of age: a double-blind, randomized clinical trial study. *J Dermatolog Treat* 2022; **33**:812–7.

47 Ahn SH, Yoon W, Lee SY *et al*. Effects of Lactobacillus pentosus in Children with Allergen-Sensitized Atopic Dermatitis. *J Korean Med Sci* 2020; **35**:e128.

48 Zeng J, Dou J, Gao L *et al*. Topical ozone therapy restores microbiome diversity in atopic dermatitis. *Int Immunopharmacol* 2020; **80**:106191.

49 Fang Z, Lu W, Zhao J *et al*. Probiotics modulate the gut microbiota composition and immune responses in patients with atopic dermatitis: a pilot study. *Eur J Nutr* 2020; **59**:2119–30. Erratum in: *Eur J Nutr* 2021; **60**:4659.

50 Jeong K, Kim M, Jeon SA *et al*. A randomized trial of Lactobacillus rhamnosus IDCC 3201 tyndallizate (RHT3201) for treating atopic dermatitis. *Pediatr Allergy Immunol* 2020; **31**:783–92.

51 Pazukhina E, Andreeva M, Spiridonova E *et al*. Prevalence and risk factors of post-COVID-19 condition in adults and children at 6 and 12 months after hospital discharge: a prospective, cohort study in Moscow (StopCOVID). *BMC Med* 2022; **20**:244.

52 Cantisani C, Chello C, Grieco T *et al*. Cutaneous Reactions to COVID-19 Vaccines in a Monocentric Study: A Case Series. *J Clin Med* 2022; **11**:3811.

53 Rizzi M, Patrucco F, Trevisan M *et al*. Baseline plasma SARS-CoV-2 RNA detection predicts an adverse COVID-19 evolution in moderate to severe hospitalized patients. *Panminerva Med* 2022. https://doi.org/10.23736/S0031-0808.22.04705-X.

54 Roy S, Iktidar MA, Chowdhury S *et al*. Prevalence of dermatological manifestations due to face mask use and its associated factors during COVID-19 among the general population of Bangladesh: A nationwide cross-sectional survey. *PLoS One* 2022; **17**:e0269922.

55 Brindisi G, Di Marino VP, Olivero F *et al*. Effects of COVID-19 lockdown on weight in a cohort of allergic children and adolescents. *Ital J Pediatr* 2022; **48**:88.

56 Uday U, Bethineedi LD, Hasanain M *et al*. Effect of COVID-19 on air pollution related illnesses in India. *Ann Med Surg (Lond)* 2022; **78**:103871.

57 Merzon E, Weiss M, Krone B *et al*. Clinical and Socio-Demographic Variables Associated with the Diagnosis of Long COVID Syndrome in Youth: A Population-Based Study. *Int J Environ Res Public Health* 2022; **19**:5993.

58 Liu H, Zhang K, Liu P et al. Improvement Effect of Bifidobacterium animalis subsp. lactis MH-02 in Patients Receiving Resection of Colorectal Polyps: A Randomized, Double-Blind, Placebo-Controlled Trial. *Front Immunol* 2022; **13**:940500.

59 Carucci L, Nocerino R, Paparo L et al. Therapeutic effects elicited by the probiotic Lacticaseibacillus rhamnosus GG in children with atopic dermatitis. The results of the ProPAD trial. *Pediatr Allergy Immunol* 2022; **33**:e13836.

60 Kamil RZ, Murdiati A, Juffrie M et al. Gut Microbiota Modulation of Moderate Undernutrition in Infants through Gummy Lactobacillus plantarum Dad-13 Consumption: A Randomized Double-Blind Controlled Trial. *Nutrients* 2022; **14**:1049.

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FIGURE LEGENDS

Figure 1. CONSORT diagram.

Figure 2. Fitted values and differences of the SCORAD between groups.

Figure 3. Percentage of patients and differences of the IGA score between groups.

Table 1. Baseline characteristics of the participants by group

	Placebo N = 35	Probiotic N = 35	Overall N = 70
Age (years); Mean (SD)	8.40 (3.77)	8.43 (3.28)	8.41 (3.51)
Gender (Female); N (%)	24 (68.6%)	22 (62.9%)	46 (65.7%)
Weight (kg); Mean (SD)	31.7 (14.3)	35.3 (15.3)	33.5 (14.8)
Height (cm); Mean (SD)	128 (28.9)	136 (19.0)	132 (24.6)
Age at onset of AD (years); Mean (SD)	0.882 (1.27)	0.857 (1.83)	0.870 (1.57)
Corticoids use; N (%)	18 (51.4%)	18 (51.4%)	36 (51.4%)
Antihistamines use; N (%)	12 (34.3%)	8 (22.9%)	20 (28.6%)
Topical antibiotics use; N (%)	4 (11.4%)	3 (8.6%)	7 (10.0%)
SCORAD; Mean (SD)	33.5 (7.09)	33.7 (5.02)	33.6 (6.10)
Spread; Mean (SD)	19.6 (11.7)	14.9 (8.35)	17.3 (10.3)
Intensity; Mean (SD)	6.31 (1.53)	6.64 (1.15)	6.48 (1.35)
Subjective symptoms; Mean (SD)	7.49 (3.85)	7.44 (3.52)	7.46 (3.66)
IGA; N (%)			
Almost cleared or Slight (1-2)	14 (40%)	8 (22.9%)	22 (31.4%)
Moderate or Severe (3-4)	21 (60%)	27 (77.1%)	48 (68.6%)

SD: Standard Deviation; AD: Atopic Dermatitis; SCORAD: Scoring of Atopic Dermatitis; IGA: Investigator Global Assessment.

Table 2. SCORAD subcomponents fitted values and differences between groups.

SCORAD subcomponent	Time	Placebo	Probiotic	Difference	P-value
Value A (Spread)	Baseline	19.64	14.93	-4.72 (-0.13 to 9.57)	0.06
	Week 6	7.39	7.32	-0.07 (-2.84 to 2.70)	0.96
	Week 12	5.97	3.41	-2.56 (-5.25 to 0.13)	0.06
Value B (Intensity)	Baseline	6.31	6.64	0.33 (-0.97 to 0.32)	0.31
	Week 6	4.10	3.91	-0.19 (-1.06 to 0.67)	0.65
	Week 12	4.19	2.92	-1.27 (-2.43 to -0.1)	0.03
Value C (Subjective symptoms)	Baseline	7.49	7.44	-0.04 (-1.72 to 1.80)	0.96
	Week 6	3.35	3.15	-0.21 (-1.79 to 1.37)	0.79
	Week 12	3.11	2.58	-0.53 (-2.09 to 1.02)	0.49

Table 3. Comparison of the proportions of days using topical corticosteroids per the number of days in the study

Time interval	Group	Days of use	Days in study	Proportion	Difference	P-value
Baseline to Week 6	Placebo	210	1522	0.138	-0.020 (-0.006 to 0.046)	0.13
	Probiotic	231	1460	0.158		
Week 6 to Week 12	Placebo	209	1378	0.152	-0.052 (-0.078 to -0.027)	<0.001
	Probiotic	136	1370	0.099		
Baseline to Week 12	Placebo	419	2900	0.144	-0.015 (-0.033 to 0.003)	0.11
	Probiotic	367	2830	0.130		

Table 4. Comparison of the grams of topical corticosteroids administered by group in the specified interval

Time interval	Group	Median (SD)	P-value
Baseline to Week 6	Placebo	3.0 (11.2)	0.67
	Probiotic	3.0 (20.6)	
Week 6 to Week 12	Placebo	4.0 (14.1)	0.02
	Probiotic	0.0 (5.6)	
Baseline to Week 12	Placebo	6.0 (21.6)	0.27
	Probiotic	5.0 (21.2)	

Table 5. Comparison of the number of adverse events by group

Type	Placebo	Probiotic	P-value
Gastrointestinal	1	5	0.22
Gastric virus	3	6	0.51
Neurological	9	4	0.27
Muscular	2	1	1
Influenza	8	2	0.10
Fever	2	4	0.71
Respiratory, eyes	12	6	0.25
Allergy, skin	6	8	0.79
Laryngology	3	3	1
Other	8	5	0.58
TOTAL	54	44	0.34

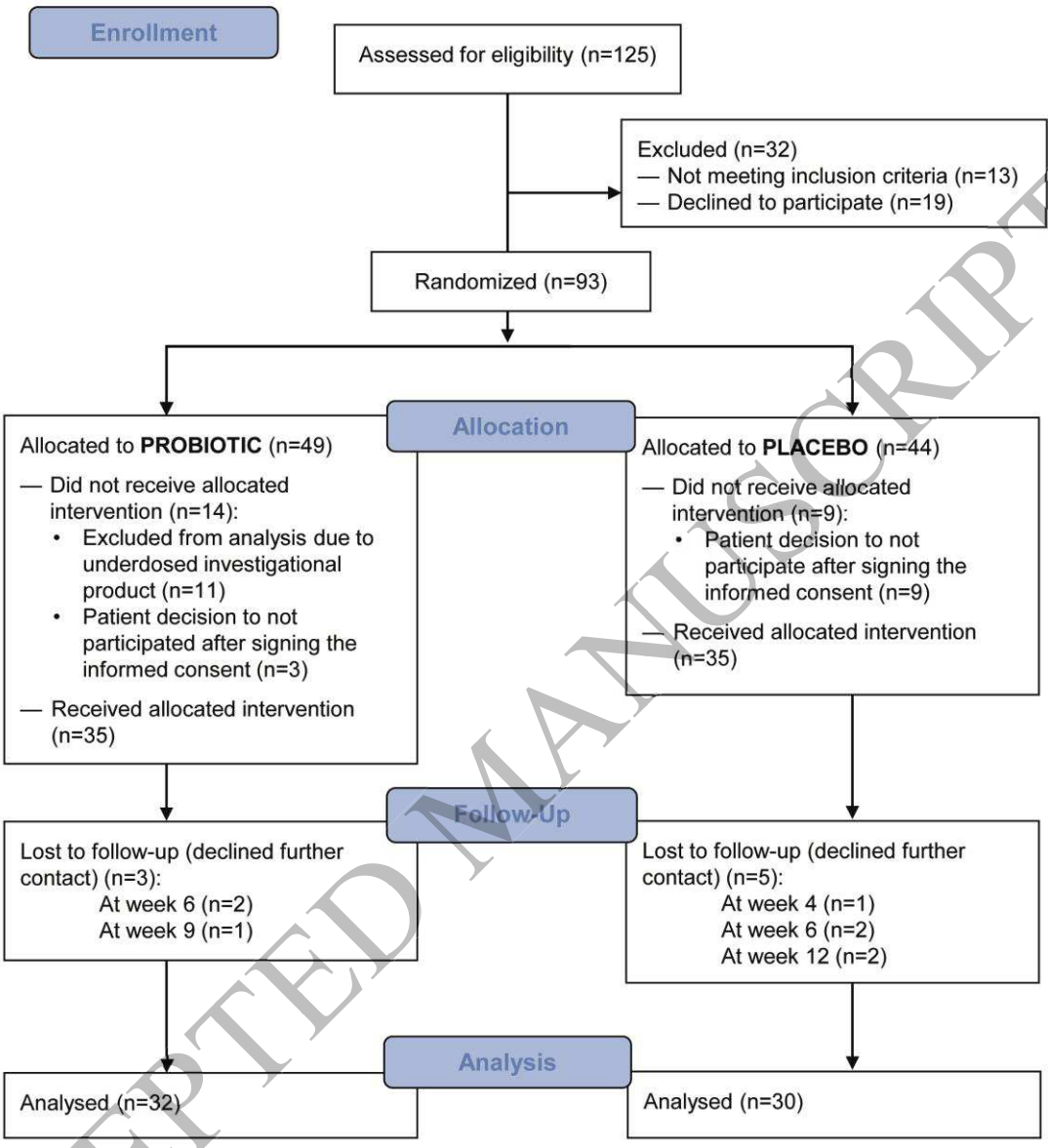
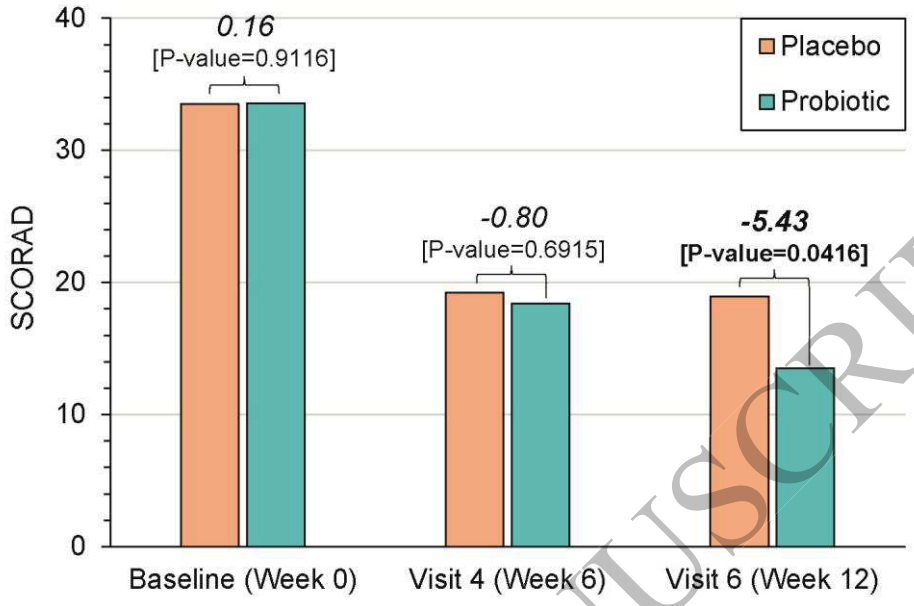


Figure 1
150x161 mm (0.7 x DPI)



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2 **Figure 2**
3 **131x89 mm (0.7 x DPI)**

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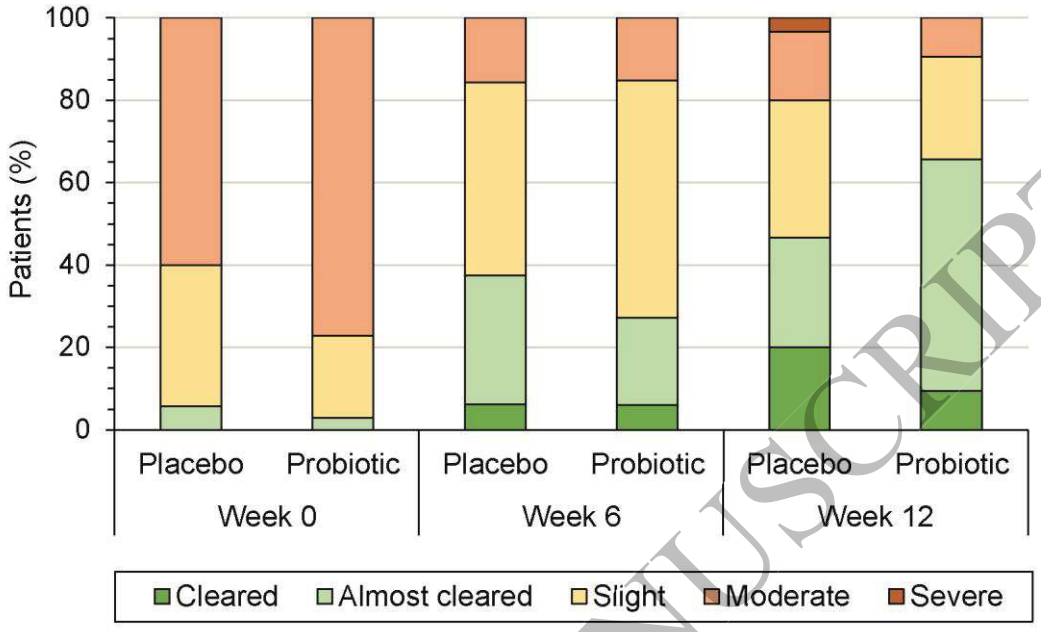

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2 **Figure 3**
3 **148x95 mm (0.7 x DPI)**