

## Preliminary Results on Clinical Effects of Probiotic *Lactobacillus salivarius* LS01 in Children Affected by Atopic Dermatitis

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**Goals:** The goal of this study was to evaluate the clinical efficacy of an intake of *Lactobacillus salivarius* LS01 (DSM 22775) for the treatment of atopic dermatitis (AD) in children.

**Background:** AD is an inflammatory and pruritic chronic relapsing skin disorder with multifactorial etiopathology. Some evidence suggests that probiotics may improve AD by modulating the immune system and the composition of intestinal microbiota.

**Study:** A total of 43 patients aged from 0 to 11 years were enrolled in the study (M/F ratio = 1:1) and treated with the probiotic strain *L. salivarius* LS01. Clinical efficacy of probiotic treatment was assessed from baseline by changes in itch index and in the objective SCORAD/SCORAD index.

**Results:** Patients being given probiotic treatment showed a significant improvement in clinical parameters (SCORAD and itch values) from baseline. The reduction in SCORAD and itch index observed after 4 weeks of treatment also persisted after the cessation of probiotic supplementation.

**Conclusions:** *L. salivarius* LS01 seems to be able to improve the quality of life of children affected by AD and, as a consequence, it may have promising clinical and research implications.

**Key Words:** atopic dermatitis, probiotics, immune system, gut microbiota

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Atopic dermatitis (AD) is a chronic inflammatory skin disease with multifactorial etiopathology, which is most prevalent in childhood.<sup>1</sup> Normally, childhood AD appears in the first 5 years of life, with about 60% of cases appearing between 0 and 6 months.<sup>2</sup> The prevalence assessment of AD is complicated due to the polymorphism characterizing the disease and the absence of pathognomic elements specific for diagnosis.<sup>3</sup> Patients with AD often experience embarrassment from skin lesions, and severe disease can adversely affect social interactions<sup>4</sup>; moreover, negative influences have also been observed in families, such as frustration, concerns, fears

about the disease, self-blame, and disappointment.<sup>2</sup> Several elements contribute to the development of AD, such as genetics, immunologic, markers and environmental factors. In particular, the decreased microbial exposure due to urbanization, the intensive use of antibiotics and vaccines, and improved infant sanitation lead to the increased development of AD.<sup>5</sup> Exposure of infants to pets and daycare environments is helpful for the introduction of positive challenges, including various nonpathogenic bacteria, which may enhance protective immunity against allergic disorders.<sup>6</sup> It has been observed that the barrier defects in AD seem to go beyond the skin, involving the intestinal mucosa,<sup>7</sup> where indigenous intestinal bacteria contribute to stabilizing intestinal permeability and mucosal barrier function.<sup>8,9</sup> The immune system of neonatal infants is not completely developed and is disposed to an immature T-helper type Th2-dominant state. Infants undergo environment-driven maturation to establish a balance between Th1, Th2, Th17, and regulatory T-cell (Treg) responses. It has been demonstrated that appropriate microbial stimulation in early life contributes to the establishment of a balanced immune system.<sup>10</sup> In recent years, there has been great interest in the use of probiotics to improve human health. Probiotic microorganisms have already been used in patients affected by irritable bowel syndrome and they seem able to modulate mucosal immune responses, leading to a reduction in gastrointestinal inflammation.<sup>7</sup> Furthermore, Roudsari and colleagues highlighted the beneficial role of ingested probiotic bacteria both on healthy and diseased skin. Their results indicate that oral consumption of probiotic microorganisms might reduce skin sensitivity, supporting the skin's immune function.<sup>11</sup> Probiotics have also been found to modulate specific and nonspecific immune responses to allergens by affecting phagocytosis and the production of proinflammatory cytokines and IgA.<sup>12-18</sup> The aim of this study was to evaluate the efficacy of oral administration of *Lactobacillus salivarius* LS01 (DSM 22775) on quality of life and clinical course in children affected by moderate and severe AD.

### MATERIALS AND METHODS

#### Patients and Study Design

A total of 43 patients aged from 0 to 11 years (M/F ratio = 1:1) with AD were recruited at the Paediatric Allergy Unit of the Hospital of Spoleto and Foligno and at Paediatric clinics involved in this study. Enrollment was carried out from December 2012 to February 2013.

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The authors declare that they have nothing to disclose.

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Patients took 2 sachets/day of a lyophilized form of *L. salivarius* LS01 (DSM 22775) ( $1 \times 10^9$  CFU/sachet) for 8 weeks, and 1 sachet/day for the following 8 weeks. Subjects could use emollients and, only if strictly needed, they could be subjected to topical steroid therapy according to the specialist's advice. Patients were visited at the beginning of the study (T0), and every 4 weeks during the probiotic treatment (T4, T8, T12, and T16). Finally, they were visited 4 weeks after the end of treatment (T20). Patients with rhinoconjunctivitis and/or acute asthma, chronic and infectious diseases, and hypersensitivity to any components contained in the probiotic sachets were excluded from the study. Moreover, in the last month before enrollment, patients were required to avoid taking probiotics, systemic steroids, systemic antihistamines, and immunomodulatory drugs. The study was approved by the Local Ethics Committee, and all patients gave their written informed consent when assessed for eligibility.

### Symptoms Score

The diagnostic criteria of AD were confirmed according to clinical guidelines published in 2004.<sup>19</sup> Clinical severity was evaluated using the objective SCORAD and the SCORAD index, one of the most validated scoring systems.<sup>20</sup> This system considers both objective signs (severity and extension) and subjective signs (pruritus and loss of sleep). The range of the SCORAD score is 0 to 80, and eczema was graded as mild (SCORAD score 0 to 15), moderate (SCORAD score 16 to 40), or severe (SCORAD score > 40). Furthermore, the itch intensity has also been evaluated.

### RESULTS

A total of 15 dropouts has been recorded. No adverse events were reported. SCORAD and objective SCORAD values were reported separately in the results. Patients being given probiotics showed a significant reduction in SCORAD during probiotic treatment ( $P = 0.001$ ) (Fig. 1). After 4 weeks, a significant decrease in SCORAD value was observed, and this continued until the end of treatment. The aforementioned reduction persisted also after suspension of treatment (Fig. 1). Itch intensity also showed a significant decrease after administration of probiotics to patients and this reduction persisted after suspension of treatment (Fig. 2).

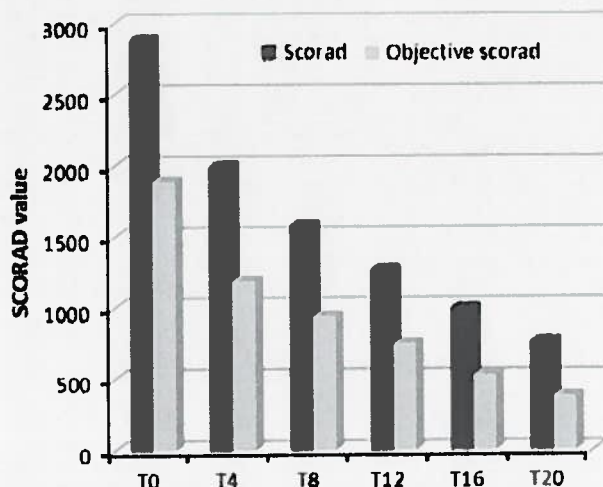


FIGURE 1. SCORAD and objective SCORAD index.

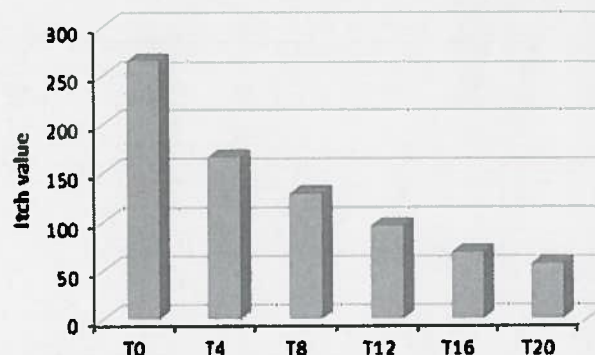


FIGURE 2. Itch index.

### DISCUSSION

The prevalence of AD has been increasing in industrialized countries as a result of western lifestyle and environmental change.<sup>21</sup> Recent knowledge about the role of skin barrier defects in initiating and perpetuating skin inflammation has led to a significant change in our understanding of the pathoetiology of AD.<sup>7</sup> In our study, the administration of the probiotic strain *L. salivarius* LS01 to children affected by moderate or severe AD led to a significant decrease in SCORAD/objective SCORAD index. This reduction was observed after 4 weeks of probiotic administration and it was accompanied by a significant reduction in itch intensity. Our results confirmed a previous study in which probiotic treatment was able to improve the extent and severity of AD in young children with moderate or severe disease.<sup>22</sup> Isolauri and colleagues highlighted the beneficial role of probiotics in children with moderate AD. Indeed, probiotics, such as *Lactobacillus rhamnosus* GG or *Bifidobacterium lactis*, were able to accelerate the complete resolution of the disease in the group receiving probiotics, compared with subjects who did not receive probiotic microorganisms.<sup>23</sup> Moreover, a recent study demonstrated that treatment with *L. salivarius* LS01 was able to positively modify clinical and immunologic status and Dermatology Life Quality in a group of patients affected by moderate or severe AD. The probiotic treatment led to a modulation of the immune system and to a rebalancing of altered intestinal microbiota, reducing the intestinal staphylococcal load in patients after 16 weeks of treatment, suggesting that the manipulation of intestinal flora with the *L. salivarius* LS01 strain could help to prevent the emergence of allergy symptoms.<sup>24</sup> The ability of *L. salivarius* LS01 to modulate the cytokine profile has already been described by Drago et al<sup>25</sup> by means of an in vitro model. Indeed, *L. salivarius* has been shown to promote a sustained increase in Th1 cytokine production, leading simultaneously to a significant decrease in Th2 response. Iemoli et al<sup>7</sup> showed that treatment with probiotic microorganisms was able to improve not only the SCORAD index, but also microbial translocation in the gastrointestinal tract. Indeed, the authors observed a significant reduction in microbial translocation during and after probiotic treatment. These results indicate that microbial translocation is actively involved in the pathogenesis of AD and that recovery of gut barrier function may improve AD clinical outcome.<sup>7</sup> Furthermore, our data showed that the benefits observed in children who were given the probiotic strain persisted 1 month after the supplementation ceased, probably due to permanent changes in the fecal flora of AD subjects. The exact mechanisms by which probiotics may improve AD are still unclear, but it may be

possible that their action is mediated by immunologic effects initiated in the gastrointestinal mucosa. Indeed, specific input from the fecal flora to the innate immune system is essential for the establishment and maintenance of mucosal immune tolerance. In particular, the Toll-related proteins TLR2, TLR4, and TLR9 recognize specific microbial components, inducing the production of T-helper 1 (Th1) cytokines through a process dependent on nuclear factor- $\kappa$ B (NF- $\kappa$ B). As a consequence, the increase in Th1 production mediated by probiotic strains may lead to a reduction of Th2-mediated allergy, rebalancing the Th1/Th2 ratio.<sup>26</sup> *L. salivarius* LS01 has already been shown to promote differentiation of Th lymphocytes (Th-0) toward Th1 subsets, acting on B cells to inhibit switching to IgE and prevent proliferation of Th2 lymphocytes.<sup>24</sup> However, the encouraging results of our study were in contrast to data by Brouwer et al,<sup>27</sup> which found no statistically significant effect of probiotic supplementation on SCORAD, inflammatory parameters, and cytokine production in infants with AD. These negative results were probably due to the probiotic strains used in the study, which had a limited effect on clinical parameters and cytokine expression.<sup>27</sup> Moreover, not all cases of atopic diseases in children share the same pathogenesis and this may influence the action of the probiotic microorganisms used in the therapy. In conclusion, our data showed a remarkable efficacy of probiotic *L. salivarius* LS01 (DSM 22775) treatment on children affected by moderate and severe AD. Moreover, the beneficial effects of probiotic treatment persisted after the cessation of supplementation. A double-blind study is needed to confirm these preliminary results and to fully investigate the clinical, immunologic, and microbiological effects of treatment with *L. salivarius* LS01 on pediatric patients affected by AD.

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**Obiettivi:** L'obiettivo di questo studio è stato quello di valutare l'efficacia clinica data dalla somministrazione di *Lactobacillus salivarius* LS01 (DSM 22775) per il trattamento della dermatite atopica (AD) nei bambini.

**Background:** AD rappresenta una patologia cutanea, ad andamento cronico/ recidivante, infiammatoria, pruriginosa della pelle con eziopatologia multifattoriale. Alcune evidenze scientifiche suggeriscono l'efficacia dei probiotici nel migliorare AD, con effetto di modulazione sul sistema immunitario e sulla composizione della flora intestinale.

**Studio:** Un totale di 43 pazienti di età compresa tra 0 a 11 anni sono stati arruolati nello studio (rapporto M / F = 1: 1) e trattato con il ceppo probiotico *L. Salivarius* LS01. L'efficacia clinica del trattamento probiotico è stato valutata tramite le variazioni dell'indice del prurito e tramite la valutazione dell'indice SCORAD e SCORAD oggettivo.

**Risultati:** I pazienti trattati con LS01 hanno mostrato un significativo miglioramento dei parametri clinici di SCORAD, SCORAD oggettivo e indice del prurito. La riduzione dello SCORAD edell' indice di prurito è stata osservata anche dopo 4 settimane dal termine del trattamento con il probiotico.

**Conclusioni:** *L. salivarius* LS01 sembra essere in grado di migliorare la qualità della vita dei bambini affetti da AD e, di conseguenza, può avere implicazioni cliniche e di ricerca promettenti.

Parole chiave: dermatite atopica, probiotici, il sistema immunitario, intestino microbiota

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