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Original Article

Therapeutical efficacy of immunobiotics in patients with newly diagnosed rheumatoid arthritis

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Abstract

Introduction: Rheumatoid arthritis (RA) remission remains a key treatment goal, but remission rates vary. Emerging evidence suggests that gut microbiota modulation via probiotics may influence systemic inflammation and improve outcomes in RA. The objective was to evaluate the effects of adjunctive probiotic supplementation on clinical outcomes in patients with newly diagnosed RA receiving conventional disease-modifying antirheumatic drugs (cDMARDs) over 12 months.

Methodology: In this randomized, placebo-controlled trial, 100 patients with newly diagnosed RA were assigned to receive either probiotics containing *Lactobacillus casei* BLn2401, *Lactobacillus salivarius* BL2201, and *Bifidobacterium breve* BL3406 plus cDMARDs (experimental group); or cDMARDs alone (control). Clinical outcomes including disease activity score using 28 joints (DAS28), inflammatory markers (C-reactive protein, CRP; erythrocyte sedimentation rate, ESR), pain (visual analogue scale, VAS), functional disability (health assessment questionnaire, HAQ), and RA quality of life (RAQoL) questionnaire were assessed at baseline and follow-up. Remission rates and corticosteroid use were evaluated.

Results: The probiotic group demonstrated faster and more sustained reductions in DAS28, CRP, ESR, pain, and disability scores; compared to controls. The probiotic group achieved near-remission (DAS28 2.3 ± 0.4) by 12 months, while the control group reverted to baseline disease activity. Probiotic use was the independent predictor of remission or low disease activity (HR = 2.703, $p < 0.001$). Patient-reported quality of life improved significantly, and corticosteroid dependence decreased in the probiotic group.

Conclusions: Adjunctive probiotic supplementation with specific strains may enhance clinical outcomes, reduce inflammation, and increase remission rates in early RA, supporting probiotics as a safe, accessible adjunctive therapy.

Key words: rheumatoid arthritis; immunobiotic; *Lactobacillus casei* BLn2401; *Lactobacillus salivarius* BL 2201; *Bifidobacterium breve* BL 3406.

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Introduction

Rheumatoid arthritis (RA) is a chronic progressive autoimmune disease which is characterized by inflammation of symmetric joints and can also cause extra articular manifestation, affecting the eye, skin, nervous system, heart, lung, gastrointestinal tract, and kidney [1]. RA is the most common type of the chronic arthritis with a prevalence of 0.4–1.3% of the population, and the women are affected 2–3 times more often [2].

Diagnosis of RA is made on the basis of patient's symptoms; physical examination; laboratory markers such as elevated values of C reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and the presence of autoantibodies such as rheumatoid factor (RF) and anti-citrullinated protein antibody (ACPA); radiography; ultrasound; and magnetic resonance imaging (MRI). Early diagnosis is very important because it leads to timely initiation of therapy management which results in minor joint damage and slowing progression of the disease [3].

Treatment of RA includes pharmacological (non-steroidal anti-inflammatory drugs, NSAIDs), glucocorticoids, and disease-modifying anti-rheumatic drugs (DMARDs)) and indispensable non-pharmacological methods (physical activity, smoking cessation, weight loss, balanced diet) [4]. NSAIDs are symptomatic therapy so they can reduce pain and swelling [5]. Glucocorticoids are highly potent anti-inflammatory drugs that are used to rapidly reduce inflammation and bridge the time until DMARDs reach their full effect. DMARDs are drugs which prevent disease progression and often combine with short-term glucocorticoids; so they are divided into conventional synthetic DMARDs (methotrexate (MTX), leflunomide, hydroxychloroquine, and sulfasalazine)),

biologic DMARDs (TNF-α inhibitors, IL-6 inhibitors, B cell depleting anti-CD20 antibodies), and target synthetic DMARDs (JAK-inhibitors) [6].

Recently, the effect of probiotics in the treatment of RA has been increasingly investigated because gut microflora seemed to be an important factor in the pathogenesis of RA. Patients with RA have enhanced gut permeability due to gastrointestinal (GI) tract inflammation enabling food antigens and some potentially dangerous pathogens to enter the bloodstream. Furthermore, studies discovered that antibodies to such antigens were found to be increased in individuals with RA, resulting in the immunological complex circulating in blood vessels [7].

Probiotics are defined as "living microorganisms that show health benefits when provided in suitable amounts" The role of probiotics is to establish balance between beneficial and harmful bacteria in the gut, to diminish bacteria causing allergies, and to revitalize gut microbiota after infections and/or antibiotics treatment [8]. The anti-inflammatory and immunomodulatory role of probiotics in RA is still being researched. Bungau *et al.* proposed four domains of probiotics' beneficial effects in RA. Firstly, they emphasized the role of the control T helper (Th) and T reg cell activities in the pathogenesis of RA, as well as the loss of the immunological tolerance. The significance of Th1–Th17 response is essential in the early phases of the development of RA. In experimental immunological illnesses such as RA, probiotic microorganisms (*Lactobacillus casei*) have been demonstrated to generate a T reg immune response via the forkhead box transcription factor FOXP3 and to enhance the suppressive function of pre-existing Tregs, leading to increased production of anti-inflammatory cytokines and decreased production of pro-inflammatory cytokines [9]. Secondly, they explained that the probiotics compete with pathogenic bacteria for nutrients and therefore reduce the possibility of pathogenic bacteria to adhere to the intestinal wall. In addition, they change the mucosal-linked immune process via immunological interaction. For example, mucin is degraded by Bacteroides leading to increased permeability of intestine. It was found that patients with RA have decreased number of beneficial bacteria in their intestine leading to higher mucin degradation. Thirdly, probiotics could have antibacterial features via improved localized secretion of IgA inflammatory cells to the pathogen affecting pathogenic bacteria reproduction and decreasing production of cytokines such as IL-12, and TNF-α without altering regulated factors such as IL-10 and tissue growth factor beta (TGF-β) [10]. Finally, they proposed that probiotics containing *Lactobacillus* and *Bifidobacterium* produce short-chain fatty acids (SCFA) that have an anti-inflammatory effect and they modulate the immune system through direct interaction with immune cells by stimulating the production of anti-inflammatory cytokines and improve the function of regulatory T cells [11,12].

The aim of this study was to investigate the effect of probiotics on the disease activity in patients with RA. The secondary aim was to compare the tolerability of conventional DMARDs regarding the use of probiotics.

Methodology

This was a single center, double-blinded, placebo-controlled randomized clinical trial (RCT) approved by the local Ethics Committee. The inclusion criteria were: diagnosis of RA according to the 2010 American College of Rheumatology (ACR) / European Alliance of Associations for Rheumatology (EULAR) classification criteria and treatment onset (MTX and/or antimalarials and/or corticosteroids). Patients were newly diagnosed with RA and had active disease at enrollment, defined as DAS28 > 3.2, despite recent initiation of treatment. The exclusion criteria were: age under 20 or above 80 years, other inflammatory arthritides (psoriatic arthritis, ankylosing spondylitis, reactive arthritis, crystal arthropathies) or systemic connective tissue diseases (SLE, Sjögren's syndrome, systemic sclerosis, myositis), treatment with leflunomid, sulfasalazine, biologic or JAK inhibitor therapy, history of radio or surgical synovectomy, *Helicobacter pylori* infection, gastrointestinal bleeding, history of gastric or duodenal ulcers, pancreatitis, history of surgical procedures in gastrointestinal system, any serious infection, inborn or acquired immunodeficiency, liver insufficiency, renal insufficiency, hematologic disorders, history of malign tumors, psychiatric disorders, and active or past treatment with probiotics that lasted longer than 7 days in past 6 months. Every patient had to sign an informed consent before participating in the study.

The study included 100 patients randomized into 2 groups. Randomization was performed using a computer-generated random sequence in a 1:1 allocation ratio. Group A (experimental group) consisted of 50 patients with RA and treated with conventional synthetic disease-modifying antirheumatic drugs (cDMARDs), followed with daily use of prescribed probiotics containing 5 billion *Lactobacillus casei* BLn2401, *Lactobacillus salivarius* BL2201, and *Bifidobacterium breve* BL3406 — 1

capsule per day. Group B consisted of 50 patients with RA who are treated with cDMARDs and placebo instead of probiotics. The patients were matched by age, gender, and socioeconomic status. Hand and foot erosions were evaluated by radiographs at baseline, interpreted by two blinded radiologists. The patients were followed up for 1 year. After the randomization there were 3 visits every 3 months with clinical, laboratory, and patients' reported measures (Table 1).

At each scheduled visit the patients were asked if they agreed to continue the study. After full body examination, the patients filled out the following questionnaires:

1. The Visual Analogue Scale (VAS), which is a pain rating scale that serves as a measure of pain intensity. The score is determined using a 10-cm ruler by measuring the distance in mm with the following cut points: no pain (0–4 mm), mild pain (5–44 mm), moderate pain (45–74 mm), and severe pain (75–100 mm).

2. The Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-Fatigue) scale to assess fatigue. This self-reported questionnaire consists of 13 items evaluating tiredness, impact on daily activities, and functional ability over the past 7 days. Each item is scored on a 5-point Likert scale (0 = not at all, to 4 = very much), resulting in a total score ranging from 0 to 52, where higher scores indicate less fatigue (better status) and lower scores indicate greater fatigue.

3. The rheumatoid arthritis quality of life (RAQoL) questionnaire to evaluate quality of life. This questionnaire has 30 questions where scores below 16 indicate better quality of life.

4. The Health Assessment Questionnaire (HAQ) for the functionality assessment. This questionnaire has 2–3 questions in each of 8 domains of everyday life which are scored on the basis of how they were performed (0 – without difficulties, 3 – complete invalidity).

5. In addition, the patients were asked about cDMARD tolerance and onset of any side effect symptom.

After that, the patients were sent to the laboratory for hematology, biochemistry, immunology, and urine testing. Investigators used CRP from the local lab to calculate disease activity score for 28 joints (DAS28). A DAS28 score below 2.6 indicated remission, while a score above 5.1 indicated very active disease. A score between 3.2 and 2.6 indicated low disease activity, and a score between 3.2 and 5.1 was indicative for moderately active RA. Other relevant clinical and demographic information were obtained from the patients' electronic charts.

Statistical analysis to evaluate the significant differences between experimental and control groups were performed in SPSS version 26 (IBM Corp, Armonk, NY; 2019). Student's t test for normally distributed continuous variables and Mann Whitney test for continuous variables without normal distribution were performed. ANOVA was performed for repeated measures. This method evaluated changes within groups over time ('time effect') and whether the patterns of change differed between groups ('group × time interaction'). A significant interaction indicated that the treatment effect varied across time points between groups. Chi-squared test was performed for categorical variables. Kaplan Meier and Cox regression analysis were used for survival analysis. Statistical significance was considered when $p < 0.05$.

Results

This study included 100 patients with newly diagnosed RA. RA was prevalent among females (84%; 84 out of 100) compared to 16% among males (16 out of 100). The average age of the patients was 57 ± 14 years. The two groups were comparable regarding

Table 1. Flowchart of scheduled visits.

	Screening	Visit 1 (Day 1, Week 0)	Visit 2 (Day 85, Week 12)	Visit 3 (Day 169, Week 24)	Visit 4 (Day 337, Week 48)
Patient history	+	-	-	-	-
Full examination	+	+	+	+	+
Hematology (CBC, ESR)	+	+	+	+	+
Biochemistry*	+	+	+	+	+
Immunology (RF, anti-CCP At)	+	-	-	-	-
Urine analysis	+	+	+	+	+
DAS28	+	+	+	+	+
VAS	+	+	+	+	+
FACIT Fatigue	+	+	+	+	+
RAQoL	+	+	+	+	+
HAQ	+	+	+	+	+

CBC: complete blood count; ESR: erythrocyte sedimentation rate; biochemistry (CRP: C reactive protein; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; GGT: gamma-glutamyl transferase; LDH: lactate dehydrogenase; fasting glucose; urea; creatinine; uric acid). RF: rheumatoid factor; anti-CCP At: anti-cyclic citrullinated peptide antibodies; DAS28: disease activity score; VAS: visual analogue scale; FACIT: fatigue-functional assessment of chronic illness therapy – fatigue; RAQoL: rheumatoid arthritis quality of life questionnaire; HAQ: health assessment questionnaire.

Table 2. Demographic, epidemiologic and clinical characteristics of patients.

Characteristics	Experimental (A) group, N = 50, (100%)	Control (B) group N = 50, (100%)	*p* value
Gender			
Female	42 (84)	41 (82)	0.796
Male	8 (16)	9 (18)	
Age (years)	60 ± 12	58 ± 15	0.235
Smoking	30 (60)	19 (38)	0.020
Hypertension	27 (54)	22 (44)	0.235
Morning stiffness	48 (98)	47 (94)	0.617
Fatigue	32 (65.3)	26 (52)	0.220
Hand erosions due to RA	18 (36)	25 (50)	0.225
Feet erosions due to RA	12 (24)	21 (42)	0.08
Rheumatoid factor	101 ± 9.8	92 ± 8.2	0.640
Anti-CCP Antibodies	257 ± 28	341 ± 38	0.258

RA: rheumatoid arthritis; anti-CCP antibodies: anti-cyclic citrullinated peptide antibodies.

serostatus (72% vs 69%), with similar proportions of RF and ACPA, and no statistically significant differences in antibody levels between groups. Hypertension was present in almost half of the patients (49%, 49 out of 100). Regarding smoking status, 49% (49 out of 100) of patients were current or former smokers, and the experimental group included more smokers than the control group (30 vs. 19; $p = 0.02$). All patients were initially treated with a standard weekly dose of 15 mg of MTX. Tolerability of MTX was better in Group A, with no patients discontinuing therapy or switching to parenteral formulations. In contrast, 20% of patients in Group B required switching to the parenteral route due to intolerance ($p = 0.03$). Hydroxychloroquine was part of the treatment in 84% patients in the experimental group and 74% in the control group. All patients were treated with corticosteroids (according to EULAR recommendations); however, by the end of the study 40% patients in the experimental and 80% of patients in the control group were treated with corticosteroids ($p < 0.05$). Additionally, 6 out of 50 patients in Group B transitioned to biologics due to very high disease activity. Specific clinical, epidemiologic and demographic characteristics of the experimental (group A) and control (group B) groups are summarized in Table 2.

A total of two groups were followed over a 12-month period: Group A (experimental group) receiving probiotics in addition to cDMARDs, and Group B (control group) treated with cDMARDs alone. Clinical outcomes were assessed at baseline and at 3, 6, 9, and 12 months. Repeated measures analysis of variance (ANOVA) was used to evaluate changes over time and differences in trajectories between groups. The results are summarized in Table 2.

A significant time effect was observed for ESR ($p < 0.05$, $\eta^2 = 0.414$), with values decreasing substantially in Group A over the 12-month period (from 40 ± 22 mm/h to 14 ± 9 mm/h). The group × time interaction was also significant ($p < 0.05$, $\eta^2 = 0.078$), indicating a different temporal pattern between groups. Group B showed a modest early reduction in ESR followed by a rebound, ending with elevated values (35 ± 15 mm/h) at 12 months. For CRP, there was a significant main effect of time ($p < 0.05$, $\eta^2 = 0.198$), and a significant

Table 3. Longitudinal changes in clinical parameters over 12 months in patients with RA treated with cDMARDs + probiotics (Group A) versus cDMARDs alone (Group B).

Parameter	Gr	Baseline	3M	6M	9M	12M	*p* (time)	η^2 (time)	*p* (group ×time)	η^2 (group ×time)
ESR (mm/h)	A	40 ± 22	20 ± 14	16 ± 11	16 ± 10	14 ± 9	< 0.05	0.414	< 0.05	0.078
	B	47 ± 23	26 ± 19	28 ± 12	28 ± 12	35 ± 15				
CRP (mg/L)	A	28 ± 3.3	8 ± 11	7 ± 8	5 ± 5.5	4.1 ± 4	< 0.05	0.198	0.026	0.038
	B	32 ± 4.1	13 ± 1.9	8.5 ± 8	11 ± 10	25 ± 3				
DAS28	A	4.9 ± 0.8	2.8 ± 0.7	2.4 ± 0.7	2.6 ± 0.6	2.3 ± 0.4	< 0.05	0.665	0.01	0.344
	B	5.2 ± 0.6	3.4 ± 0.9	3.4 ± 0.7	4 ± 0.8	4.8 ± 0.7				
VAS (0–10)	A	9.3 ± 0.7	1.8 ± 1.3	0.6 ± 1	0.3 ± 0.6	0.2 ± 0.4	< 0.05	0.851	0.001	0.602
	B	9.5 ± 0.5	4.7 ± 2	4.7 ± 1.8	6 ± 1.6	7.8 ± 1.6				
HAQ	A	1.0 ± 0.4	0.86 ± 0.4	0.8 ± 0.4	0.8 ± 0.4	0.77 ± 0.5	0.016	0.04	0.035	0.33
	B	1.1 ± 0.5	0.9 ± 0.4	1 ± 0.6	1 ± 0.53	1.2±0.5				
RAQoL	A	12 ± 7	8.7 ± 6.5	8.16 ± 6	7.8±6.2	7.59 ± 6	< 0.05	0.125	0.01	0.056
	B	14 ± 7	10 ± 7	11 ± 6.7	12.7±7	14 ± 6.7				

RA: rheumatoid arthritis; Gr: group; M: month: ESR: erythrocyte sedimentation rate; CRP: C reactive protein; DAS28: disease activity score; VAS: visual analogue scale; RAQoL: rheumatoid arthritis quality of life questionnaire; HAQ: health assessment questionnaire. Values are presented as mean ± standard deviation. *p* (time): significance of time effect in repeated measures ANOVA. η^2 (time): effect size for time ($\eta^2 \geq 0.14$ = large). *p* (group × time): significance of interaction between group and time. η^2 (group × time): effect size for interaction. Significant group × time interaction indicates that the change over time differed between the two groups.

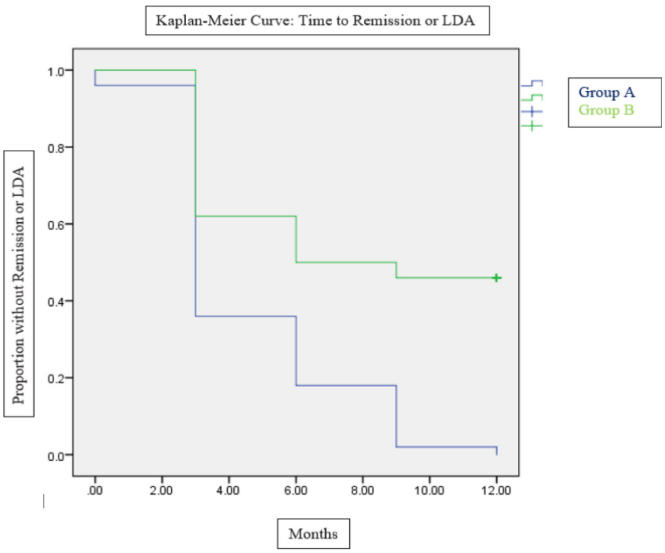
group × time interaction ($p = 0.026$, $\eta^2 = 0.038$). Group A exhibited a continuous decline in CRP levels from 28 ± 3.3 mg/L to 4.1 ± 4 mg/L, whereas Group B showed a partial reduction followed by a relapse at 12 months (25 ± 3 mg/L) (Table 3).

A highly significant time effect was observed for DAS28 ($p < 0.05$, $\eta^2 = 0.665$), as well as a strong group × time interaction ($p = 0.01$, $\eta^2 = 0.344$). Group A demonstrated a pronounced decrease in disease activity, achieving near remission (2.3 ± 0.4) by month 12. In contrast, Group B initially improved but then worsened, returning to baseline levels (4.8 ± 0.7).

Pain, assessed by VAS, showed a very strong time effect ($p < 0.05$, $\eta^2 = 0.851$) and a robust group × time interaction ($p = 0.001$, $\eta^2 = 0.602$). Group A experienced a dramatic reduction in pain from 9.3 ± 0.7 to 0.2 ± 0.4, while group B showed only partial and transient relief, with values increasing to 7.8 ± 1.6 by month 12.

There was a significant time effect for HAQ ($p = 0.016$, $\eta^2 = 0.04$) and a significant group × time interaction ($p = 0.035$, $\eta^2 = 0.33$) in the domain of functional disability (HAQ). Functional ability improved modestly in group A (1.0 ± 0.4 to 0.77 ± 0.5), whereas group B remained stable or worsened (1.1 ± 0.47 to 1.2 ± 0.54). A significant time effect was observed for RAQoL ($p < 0.05$, $\eta^2 = 0.125$), along with a significant group × time interaction ($p = 0.01$, $\eta^2 = 0.056$). Group A showed sustained improvements in quality of life (from 12 ± 7 to 7.6 ± 6.3), while group B returned to baseline levels (14 ± 6.7) after initial transient gains.

Figure 1. Kaplan-Meier survival curve for time to achieving remission or low disease activity (DAS28 ≤ 3.2) in patients treated with immunobiotics + cDMARDs (Group A) vs. cDMARDs alone (Group B) over 12 months. Survival in this context represents not yet achieving remission/low disease activity.



Kaplan-Meier analysis showed a significantly faster and higher rate of achieving remission or low disease activity in the Group A compared to the group B (log-rank test $p < 0.05$). At 12 months, nearly all patients in the experimental group reached disease remission or low disease activity, whereas a substantial proportion of patients in the control group remained in active disease ("survival" 45%). The survival curve for the group A dropped sharply in the first 6 months, indicating rapid improvement, while the control group's decline was slower and plateaued higher (Figure 1).

Cox proportional hazards regression was conducted to identify independent predictors of achieving remission or low disease activity (LDA) over a 12-month follow-up period. The model included treatment group, gender, age, smoking status, baseline DAS28 score, baseline ESR and CRP levels, and hand radiographic findings as covariates. The analysis revealed that the treatment group was the only significant independent predictor of time to remission or LDA. Specifically, patients in the experimental group receiving probiotics in addition to conventional DMARDs had a significantly higher chance of achieving remission or LDA compared to those in the control group (hazard ratio [HR] = 2.703, 95% confidence interval [CI]: 1.590–4.597, $p < 0.001$). Other variables, including gender ($p = 0.712$), age ($p = 0.222$), smoking ($p = 0.783$), baseline DAS28 ($p = 0.607$), baseline ESR ($p = 0.637$), baseline CRP ($p = 0.188$), and hand radiographic changes ($p = 0.670$), were not significantly associated with the outcome.

Discussion

In this randomized controlled trial, adding probiotics to conventional DMARD therapy significantly improved clinical outcomes in patients with newly diagnosed RA over a 12-month period. Patients receiving probiotics achieved faster and more sustained reductions in disease activity, inflammatory markers, pain, and functional disability compared to those treated with cDMARDs alone.

The most notable finding was the substantial improvement in disease activity scores (DAS28) in the probiotic group. Although both groups initially showed some improvement, only the probiotic group maintained this progress and reached near-remission by the study's end (DAS28 2.3 ± 0.4), while the control group's disease activity returned to baseline. These results align with prior studies suggesting that gut microbiota modulation can influence systemic inflammation in RA through immune regulatory

mechanisms, including enhanced regulatory T cell (Treg) activity and decreased Th17 responses [8,9].

Kaplan-Meier survival analysis further confirmed that a significantly larger proportion of patients in the probiotic group achieved remission or low disease activity faster than controls. Cox regression analysis identified probiotic use as the sole independent predictor of remission or low disease activity (HR = 2.703, $p < 0.001$).

Achieving remission in RA is the primary treatment goal, but remission rates vary widely due to diverse prognostic factors. Early diagnosis and prompt DMARD initiation, especially within the initial "window of opportunity," are strongly associated with higher remission rates [13]. Lower baseline disease activity also predicts better outcomes. Serologic status plays a key role: seronegative patients (negative for RF and ACPA) typically have a more favorable prognosis compared to seropositive individuals, who often experience more severe disease. Genetic factors, such as specific *human leukocyte antigen - DR Beta 1* (*HLA-DRB1)* alleles, also influence disease severity and treatment response. Younger age at onset and good treatment adherence further increases remission likelihood, whereas comorbidities (obesity, cardiovascular disease, depression) and lifestyle factors like smoking negatively impact remission [14]. Imaging markers of minimal baseline joint damage correlate with better long-term outcomes. Notably, traditional prognostic factors such as age, gender, smoking, radiographic changes, and baseline inflammation were not predictive in this cohort, highlighting the potential immunomodulatory impact of probiotics and the need for further studies. These multifactorial determinants emphasize the importance of individualized treatment strategies and comprehensive patient assessment to optimize remission in RA [15].

Beyond disease activity, probiotic supplementation improved patient-reported outcomes. Pain, measured by VAS, decreased dramatically in the probiotic group, whereas the control group experienced only partial and transient relief. Likewise, HAQ and RAQoL scores improved significantly in the probiotic group, indicating meaningful enhancements in daily functioning and quality of life. These findings echo prior clinical studies demonstrating the immunomodulatory effects of probiotics in RA. For example, Pineda *et al.* and Vaghef-Mehrabany *et al.* reported significant reductions in CRP and DAS28 following probiotic supplementation, consistent with the observed decreases in inflammatory markers and improved disease activity. Alipour *et al.* further showed enhanced quality of life and functional status in RA patients consuming probiotic-rich yogurt, mirroring improvements in HAQ and RAQoL scores seen in this experimental group [16–18].

Importantly, inflammatory markers such as CRP and ESR declined significantly over time in the probiotic group but not in controls, suggesting that clinical improvements paralleled objective reductions in systemic inflammation. These effects align with proposed probiotic mechanisms, including competition with pathogenic bacteria, production of SCFAs, and regulation of inflammatory cytokines such as IL-12 and TNF-α [10,11].

This study also found that fewer patients in the probiotic group required corticosteroids at 12 months, indicating better disease control and potentially reduced exposure to steroid-associated adverse effects. In addition, all patients in the probiotic group maintained MTX therapy throughout the 12-month follow-up, suggesting improved tolerability and disease control. This is clinically significant, as sustained MTX use is crucial for long-term rheumatoid arthritis (RA) management. Although direct studies on the combined effect of MTX and *Lactobacillus casei* BLn2401, *Lactobacillus salivarius* BL2201, and *Bifidobacterium breve* BL3406 in RA patients are limited, research on similar probiotic strains suggests potential benefits in modulating inflammation and improving disease activity [16]. The finding of improved MTX tolerability in the probiotic group aligns with previous studies suggesting probiotics may reduce gastrointestinal side effects and support gut barrier function, potentially enhancing treatment adherence. As MTX discontinuation or switching to parenteral forms was more common in Group B, reduced adherence may have partially contributed to worse clinical outcomes. The initial improvement in Group B likely reflects the early response to MTX and corticosteroids. The subsequent worsening may relate to reduced MTX adherence/tolerability, persistent systemic inflammation, and lack of sustained immunomodulation provided by probiotics. These findings support further investigation into the specific effects of these strains in RA management.

Strengths and limitations

The strengths of this study include its randomized, placebo-controlled design, longitudinal follow-up, and use of both clinician-reported and patient-reported outcomes. However, the limitations should be acknowledged. The single-center design may limit

generalizability. The gut microbiome composition was not analyzed, preventing direct insight into host–microbiota interactions. Lastly, adherence to probiotic supplementation was assessed by self-report, which may introduce bias.

Clinical implications and future directions

The findings support the potential role of probiotics as adjunctive therapy in early RA management. Given their favorable safety profile, accessibility, and potential to reduce disease activity and steroid use, probiotics may represent a cost-effective strategy to improve outcomes in newly diagnosed RA patients. Future studies should investigate microbiome composition and metabolite profiles to clarify mechanistic pathways and validate these results in larger, multi-center trials.

Conclusions

This randomized controlled trial demonstrates that adjunctive probiotic supplementation alongside conventional DMARD therapy significantly improves clinical outcomes in newly diagnosed rheumatoid arthritis patients over a period of 12 months. Probiotics accelerated and sustained reductions in disease activity (DAS28), inflammatory markers, pain, and functional disability; compared to cDMARD therapy alone. Importantly, probiotic use independently predicted remission and low disease activity, underscoring its immunomodulatory potential.

Moreover, probiotics enhanced patient-reported quality of life and reduced corticosteroid dependence, suggesting benefits beyond standard disease control. Although classical prognostic factors did not predict remission in this cohort, these findings highlight gut microbiota modulation as a promising therapeutic target.

Given their safety and accessibility, probiotics containing *Lactobacillus casei* BLn2401, *Lactobacillus salivarius* BL2201, and *Bifidobacterium breve* BL3406 represent a promising adjunctive strategy to optimize remission rates and improve long-term outcomes in early RA. Larger, multi-center studies incorporating microbiome analyses are warranted to confirm these results and elucidate underlying mechanisms.

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Conflict of interest

No conflict of interest is declared.

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