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Ciprofloxacin and probiotic *Escherichia coli* Nissle add-on treatment in active ulcerative colitis: A double-blind randomized placebo controlled clinical trial

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Abstract

Background and aim: Ulcerative colitis (UC) is a chronic inflammatory bowel disease. The probiotic bacterium *Escherichia coli* Nissle 1917 (EcN) has been used to maintain and induce clinical remission in UC. Our aim was to test the effect of Ciprofloxacin and/or orally administered EcN as add-on to conventional therapies in patients with active UC.
Patients and methods: Our single center double-blinded randomized placebo controlled study included patients with a Colitis Activity Index (CAI) score of at least 6. Patients were randomized to Ciprofloxacin or placebo for 1 week followed by EcN or placebo for 7 weeks. All 4 treatments were given as add-on treatments.
Results: One hundred subjects with active UC were recruited. In the per-protocol analysis we, surprisingly, found that in the group receiving placebo/EcN fewer patients, 54%, reached remission compared to the group receiving placebo/placebo, 89%, $p < 0.05$. Among patients treated with Cipro/placebo and Cipro/EcN, 78% and 66% reached remission, respectively. Furthermore, the group receiving placebo/EcN had the largest number of withdrawals, 11 of 25 (44%), compared to 15 of 75 (20%) in any of the other groups, $p < 0.05$. Indication of lack of mucosal healing was found in the group treated with placebo/Nissle, since only 4 (29%) of the 14 patients, who completed the study, reported no blood in stools at week 12 ($p < 0.02$), compared to 63%, 67% and 65% in groups treated with Cipro/Nissle, Cipro/placebo and placebo/placebo, respectively.

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Conclusions: Our data suggest that there is no benefit in the use of *E. coli* Nissle as an add-on treatment to conventional therapies for active ulcerative colitis. Furthermore, treatment with *E. coli* Nissle without a previous antibiotic cure resulted in fewer patients reaching clinical remission.

1. Introduction

Inflammatory Bowel Disease (IBD) is traditionally divided into Crohn's disease (CD) and ulcerative colitis (UC). Both CD and UC are characterized by intestinal mucosal inflammation and intermittent episodes of remission and relapse. Both diseases are characterized by an up-regulated mainly T-cell mediated immune response, which is believed to involve the intestinal microbiota. For example an exclusion of the fecal stream by ileostomy in CD will often lead to healing of the bypassed inflamed intestine, while reintroducing the intestinal microbiota will lead to reactivation of the inflammation.[1] Furthermore, antibiotics seem to have some effect in the treatment of IBD patients.[2] Numerous experiments involving IL-10 knock-out mice have shown, that these mice will develop fulminant colitis living under normal conditions, whereas they will be disease free living in germ-free surroundings.[3] Moreover, it has been demonstrated that probiotics can reduce the inflammatory damage of the intestine in IL-10 knock-out mice.[3] Among bacteria possibly involved in IBD, *Escherichia coli* have in recent years drawn increasing interest. It has been suggested that a new pathotype of *E. coli*, adherent-invasive *E. coli*, is associated with CD.[4] In several studies an increased amount of *E. coli* has been found among IBD patients compared to controls.[5,6] *E. coli* of the phylogenetic group B2 have been associated with both CD and UC,[7] and we have previously found that this *E. coli* pathotype is particularly associated with the active phase of UC.[8] Interestingly, it has been found that the probiotic *E. coli* Nissle (EcN) has an equivalent effect to mesalazine (5-ASA) in preventing disease flares in patients with UC.[9,10] Furthermore the ability of EcN to induce remission in patients with active disease was also comparable to 5-ASA.[10] Recently, it has been proven that 5-ASA does not have an antimicrobial effect on EcN,[11] therefore a possible additive effect of EcN to standard of care in UC may be plausible. In this way, it would, theoretically, be possible to improve both a possible dysbiotic driven epithelial barrier dysfunction and the overactive immunological response during UC flares. The purpose of our study was to investigate, if treatment with Ciprofloxacin for 1 week followed by therapy with *E. coli* Nissle (EcN) for 7 weeks, or either of these treatments alone, could influence the remission rate among UC patients with disease flares compared to placebo, when Ciprofloxacin and/or *E. Coli* Nissle were given as add-on treatment to standard medical therapies.

2. Materials and methods

2.1. Aims and objectives

Our primary objective was to compare the number of patients achieving remission, evaluated by clinical activity index score, CAI-score ≤ 4, among patients treated with Ciprofloxacin for 1 week and/or EcN for 7 weeks as an add-on treatment to conventional therapies. CAI-score was performed as described by Rachmilewitz, including laboratory findings, CRP and hemoglobin.[12] Secondarily, we wanted to evaluate the number of patients withdrawn from the study in the different treatment groups.

2.2. Patients

100 consecutive patients with a flare of UC followed in the Department of Gastroenterology, Hvidovre University Hospital, Hvidovre, Denmark. Specialists in gastroenterology from our department had diagnosed all included patients according to standardized criteria. All patients had a sigmoidoscopy or colonoscopy performed previously confirming their diagnosis, however, no additional endoscopies were performed as part of the current study. Patients were aged >18 years, with a CAI-score ≥ 6. An endoscopic score was not performed as part of the study protocol; however, all patients had a fecal calprotectin performed to confirm disease activity among included patients, cut off 50 mg/kg. Exclusion criteria; pregnant or breastfeeding females, known hypersensitivity to Ciprofloxacin, subjects who participated in another clinical trial, positive stool sample with any enteric pathogens, parasites or *Clostridium difficile*, current treatment with systemic corticosteroids or biologic therapy. Patients were followed for 12 weeks. All patients in our highly specialized department, serving a population of 500,000 citizens (807 registered with UC in our department), were evaluated during visits in our out-patient clinic with a CAI-score and if, this was over 6, and no exclusion criteria were present, patients were asked if they wanted to participate in our study. Only a few patients fulfilling inclusion criteria declined participation.

2.3. Study design

The study was designed as a randomized double blinded placebo-controlled study of the effect of an add-on treatment to conventional medical therapies to patients with a flare in their UC. Patients were allocated to one of four treatment groups: Ciprofloxacin (500 mg × 2 daily) for 1 week followed by EcN for 7 weeks (100 mg × 1 for 4 days followed by 100 mg × 2 daily for the rest of the period), Ciprofloxacin for 1 week followed by placebo for 7 weeks, placebo for 1 week followed by EcN for 7 weeks or placebo for 1 week followed by placebo for 7 weeks. Patients were randomly assigned, according to a randomization carried out at the central hospital pharmacy (Region HovedstadensApotek, Marielundvej 25, 2730 Herlev, Denmark) in Copenhagen, to treatment groups; 1:1:1:1, allowing 25 patients to be included in each group.

2.4. Medication

Standard medical care and therapies, and relevant changes in these based on the treating physicians' discretion, were allowed throughout the study, however, patients requiring current treatment with systemic steroids or TNF-α inhibitors were excluded from the study, topical steroids were allowed. The EcN bacteria was a bacterial preparation for oral use containing non-pathogenic *E. coli* of strain Nissle 1917 (serotype O6:K5:H1, Mutaflor 100 mg, 2.5–25 × 10^9 bacteria per capsule, Ardeypharm GmbH, Herdecke, Germany), dosage of EcN was according to the manufacturer's recommendations. The placebo preparations were manufactured and placebo and active treatments were packaged and distributed by the central hospital pharmacy (Region Hovedstadens Apotek, Marielundvej 25, 2730 Herlev, Denmark) securing double blinding, which was upheld until after data-analysis.

2.5. Follow-up

CAI-score was carried out at inclusion, after the 1st week, the 4th week and the 8th week and at the 12th week (4 weeks after completion of study medicine). CAI score was performed by the same person throughout the whole study. Possible side effects and concomitant medication were recorded simultaneously. Blood samples were collected at each visit, and stool samples were collected at week 0, 1, 8 and 12 for future research.

2.6. Statistical approach

We aimed to include 98 participants based on an effect of placebo of 0.60 and an effect of Ciprofloxacin and/or EcN of 0.85, with a power (1-β) of 80% and an alpha of .05 (two-tailed test) for intention-to-treat analysis. Kaplan–Meier curves were used to compare groups. Test of equality of survival distributions for the different clinical treatment groups was performed using the Mantel–Cox (log-rank) test. Comparison of number of with-drawn patients from each treatment group and number of patients reporting blood in stools at the end of the study was done by Fisher's exact test.

2.7. Ethical considerations

The trial was approved by the Danish Data Protection Agency (2011-41-6203), and permission for human experiments and recruitment of participants was obtained from the Scientific Ethics Committee for Copenhagen Regional Hospitals (Permission no. H-1-2009-110) and all participants gave their informed written consent. As EcN is considered a dietary supplement and not a pharmaceutical no authorization by the Danish Medicines Agency was required. The study was performed in accordance with the requirements of GCP and the Revised Declaration of Helsinki. The study was registered in www.clinicaltrials.gov (NCT01772615).

3. Results

3.1. Patient characteristics

Overall 100 patients were included in the study and randomized into four groups, 25 patients in each group, planned to receive Ciprofloxacin or placebo for 1 week, followed by *E. coli* Nissle (EcN) or placebo for 7 weeks. Study medication was given as an add-on treatment to the patient's usual medication as prescribed during their visits in our out-patient clinic or our in-hospital department. Patients in the four groups were comparable regarding age, gender, BMI, smoking habits, CRP-levels at inclusion, years since diagnosis, previous disease extension and previous treatment with immunomodulators (Tables 1a and 1b). Upon inclusion, patients in the group receiving Cipro/EcN were more frequently treated with azathioprine or 6-mercaptopurin and corticosteroid enema than in any other group, while the group receiving placebo/EcN was less often treated with these medications (Table 2). However, when looking at the whole group (50 patients) treated with EcN, medical treatments were, overall, comparable to the group (50 patients) not treated with EcN (Table 2). Disease activity at inclusion was found to be somewhat higher in the group of patients treated with Ciprofloxacin and EcN, mean CAI 10.5, compared to the other groups, Cipro/placebo, 8.9, placebo/EcN, 9.3 and placebo/placebo, 8.9 (Table 3). This difference was not significant.

3.2. Efficacy

In the per-protocol analysis more patients in the placebo/placebo group reached remission while in the study, 89%, compared to all other treatment groups. In the groups treated with Cipro/placebo and Cipro/EcN, 78% and 66% reached remission, respectively. However, in the group of patients treated with placebo/EcN the fewest patients had reached remission by the end of the study, 54%, Fig. 1. The difference between the placebo/placebo group and the placebo/EcN group was statistically significant in the per-protocol analysis

Table 1a Baseline data regarding age, gender, BMI, smoking and CRP level at the time of inclusion in the four treatment groups.

Treatment group	Age	Gender				BMI	Smoking	CRP
		Male		Female				
	Mean	Number	%	Number	%	Mean	Number	Median
Cipro/Nissle	38.4	9	36%	16	64%	25.4	6	3
Cipro/placebo	39.3	9	36%	16	64%	25.2	3	2
Placebo/Nissle	38.7	11	44%	14	56%	25.6	4	3
Placebo/placebo	38.1	9	36%	16	64%	25.3	4	2

Table 1b Baseline data in the four treatment groups regarding years with a diagnosis of ulcerative colitis, previous known maximal disease extension, and history of TNF-a or systemic prednisolone or azathioprine/5-mercaptopurine.

Treatment group	Years since UC diagnosis	Disease extension			Medical history		
		Proctitis	Leftsided	Pancolitis	TNF-α	Systemic prednisolone	Azathioprine/ 6-mercapto-purine
	Median (range)	Number	Number	Number	Number	Number	Number
Cipro/Nissle	4 (0–13)	3	13	9	3	17	14
Cipro/placebo	6 (0–45)	4	18	3	1	15	9
Placebo/Nissle	4 (0–24)	3	18	4	1	10	7
Placebo/placebo	5 (0–38)	4	14	7	4	14	11

($p < 0.05$). Even though, endoscopies were not performed as part of our study, indication of lack of mucosal healing was found in the group treated with placebo/Nissle, since only 4 (29%) of the remaining 14 patients in this group reported no blood in stools at week 12 ($p < 0.02$), compared to 63%, 67% and 65% in groups treated with Cipro/Nissle, Cipro/placebo and placebo/placebo, respectively. When looking at the intention to treat analysis, last-observation-carried-forward method, groups were ranked in the same order as in the per-protocol analysis, with more patients in clinical remission at the end of the study in the group receiving placebo/placebo, where 80% of the 25 included reached remission during the study, the next best in the group receiving Cipro/placebo, 72%, followed by the group receiving Cipro/EcN, 60%. The worst outcome was again found in the group receiving placebo/EcN, 41%. When comparing groups receiving EcN (Cipro/Nissle and placebo/Nissle), with groups not receiving EcN (Cipro/placebo and placebo/placebo), in a survival analysis, it was demonstrated that groups receiving EcN reached remission (CAI-score ≤ 4) less frequent than groups not receiving EcN, Fig. 2, $p = 0.02$. By comparing the groups receiving or not receiving Ciprofloxacin, no statistically significant difference was found at the end of the study, week 12, regarding number of patients in remission. However, the mean CAI score was the lowest in week 8 in the group of patients treated with Ciprofloxacin alone, although statistical significance was not reached. No statistically significant difference was found in time to remission between groups, when looking at patients that remained in the study through week 12.

3.3. Withdrawals and side-effects

Twenty-six patients were withdrawn from the study, the majority of these (11 of 26) from the group receiving placebo for 1 week followed by EcN for 7 weeks ($p < 0.05$), Fig. 3. In the three other treatment groups a maximum of 6 patients were withdrawn from any individual group. Overall, 11 patients were withdrawn from the study, because they required treatment with systemic prednisolone (>50 mg per day) or TNF-α inhibitors, 8 of these were from the group of patients receiving EcN, and 3 from the group not receiving EcN, this was, however, not statistically significant, $p = 0.20$. Three patients needed admission to our hospital during the 12 week observation period (two treated with Cipro/EcN, and one treated with placebo/EcN) and one of the patients treated with Cipro/EcN required a colectomy. Likewise, among withdrawn patients, 9 patients in the group of patients receiving EcN had a higher or unaltered CAI-score at withdrawal than at entry in the study compared to 2 withdrawn patients without EcN treatment, which is interesting, but not quite reaching statistical significance, $p = 0.051$. No significant differences were noted in the reported side effects among different treatment groups. During Ciprofloxacin/placebo treatment 6 patients reported side effects, 4 treated with Ciprofloxacin experienced either itching, nausea or bloating and abdominal pain and 2 with placebo experienced either nausea or bloating and abdominal pain. During the 7 weeks of EcN/placebo treatment 18 patients reported side effects, 9 treated with EcN (itching, nausea, bloating, abdominal pain) and 9 treated with placebo (nausea, bloating, abdominal pain, sensitivity towards smells).

4. Discussion

Previously, the probiotic *E. coli* strain Nissle 1917 (EcN) has been reported to maintain remission of UC in human patients and to prevent colitis in different murine models of colitis.[9,10,13] Our study, however, is the first randomized double blind study to evaluate the efficacy of orally

Table 2 Other medical treatments given at the time of inclusion in the four treatment groups.

Treatment group	Systemic-5-ASA		Topical-5-ASA		Azathioprine/ 6-mercaptopurine		Topical prednisolone	
	Number	%	Number	%	Number	%	Number	%
Cipro/Nissle	19	76%	17	68%	8	32%	9	36%
Cipro/placebo	15	60%	15	60%	6	24%	6	24%
Placebo/Nissle	16	64%	13	52%	2	8%	1	4%
Placebo/placebo	15	60%	18	72%	6	24%	1	4%

Table 3 CAI scores at study start, at weeks 1, 4, 8 and 12 in the four different treatment groups among patients, who continued in the study.

Treatment		CAI0	CAI1	CAI4	CAI8	CAI12
Cipro/Nissle	N	25	25	23	21	19
	Mean	10.52	8.24	5.74	5.38	4.10
	Minimum	6	2	0	0	0
	Maximum	20	22	15	12	14
Cipro/placebo	N	25	25	25	22	21
	Mean	8.88	7.16	4.60	2.77	4.14
	Minimum	6	0	0	0	0
	Maximum	13	12	10	8	20
Placebo/Nissle	N	25	23	18	16	14
	Mean	9.28	7.57	6.78	4.44	5.29
	Minimum	6	0	1	0	0
	Maximum	16	16	21	11	13
Placebo/placebo	N	25	25	22	20	20
	Mean	8.88	6.84	5.18	3.95	4.90
	Minimum	6	1	0	0	0
	Maximum	15	12	12	12	12

administered EcN as an add-on treatment to conventional medical therapies in relapsing UC. Although EcN was originally isolated in 1917, the underlying mechanism of its effect in various intestinal diseases, including UC is not fully understood. EcN's assumed beneficial effect could be explained by EcN's lack of specific virulence factors (i.e., alpha-hemolysin, P-fimbrial adhesins, and the semirough lipopolysaccharide phenotype) combined with the expression of fitness factors such as microcins, different iron uptake systems, adhesins, and proteases.[14] Theoretically, it is possible that EcN suppresses or eradicates other more harmful bacteria involved in the pathogenesis of UC. Interestingly, EcN is also of the phylogenetic group B2 thus sharing many traits with the pathotypes of *E. coli* associated with IBD.[15] An intriguing mechanism in the prophylactic effect of EcN in IBD could be that EcN is able to eradicate the IBD-associated *E. coli* or that EcN is able to hinder re-infection with these *E. coli* pathotypes. Our hypothesis was, therefore, that treatment with EcN would result in more patients in remission at the end of our study, or secondarily that patients treated with EcN would reach remission faster, without differences in withdrawal rates. Surprisingly, we observed that significantly fewer patients treated with EcN reached remission, and that significantly more patients treated with EcN withdrew from the study. A cautious interpretation is reasonable regarding the study power, since our patients treated with 7 weeks of EcN or placebo, were also randomized to an initial treatment with 1 week of Ciprofloxacin or placebo. The

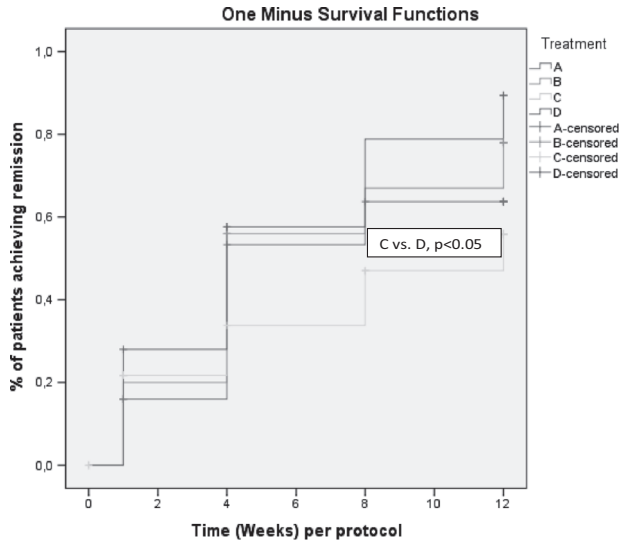

Figure 1 Patient reaching remission (per-protocol-analysis) during 12 weeks of follow-up in patients treated with Cipro/EcN (A), with Cipro/placebo (B) or with placebo/EcN (C) as add-on treatment compared to patients (D) treated with placebo/placebo.

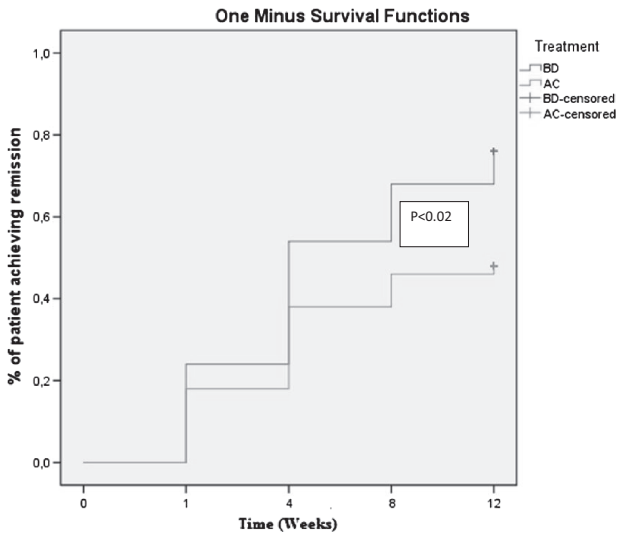

Figure 2 Patient reaching remission (intention-to-treat analysis) during 12 weeks of follow-up in patients treated with EcN (AC) as add-on treatment compared to patients (BD) not treated with EcN.

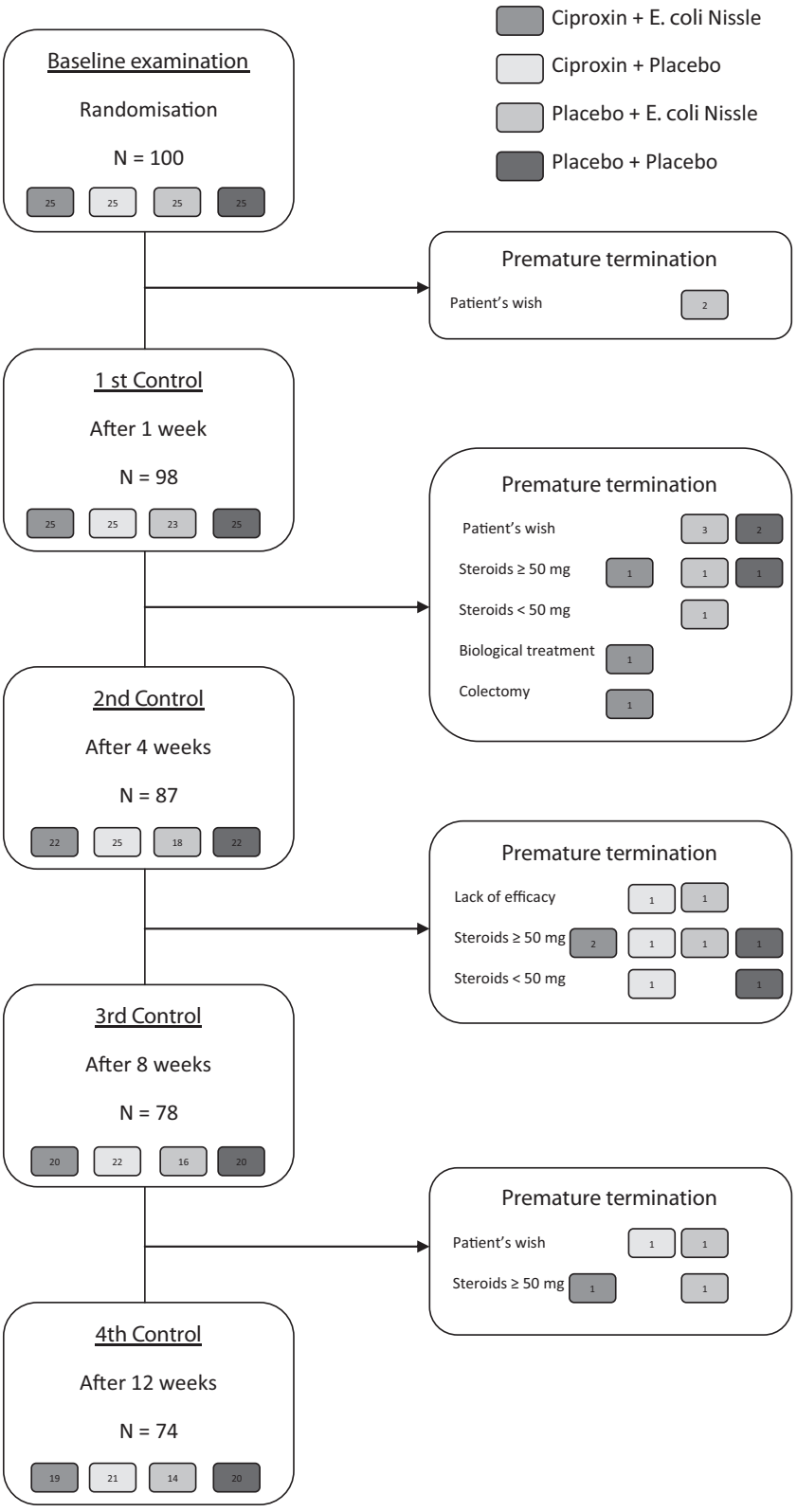

Figure 3 Flowchart of patient inclusion and withdrawals.

possible effect of Ciprofloxacin treatment should, however, have been the same in groups receiving or not receiving EcN, since half of the patients in both groups were initially treated with Ciprofloxacin. Our approach, interestingly, revealed that patients treated with EcN as add-on treatment without an initial antibiotic cure, did significantly worse also in the

per-protocol analysis, than patients receiving only placebo/placebo treatment alongside the standard of care medication given to all patients participating in this study. We do not believe that side effects due to EcN treatment could explain both fewer patients reaching a CAI score indicating remission and the higher number of withdrawals, since no differences were seen in frequency of reported side effects between patients treated with EcN and patients treated with placebo. The fact that fewer patients were treated with Azathioprine and/or steroid enemas upon inclusion in the group treated with placebo/EcN, could raise some concern regarding our results, however, treating physicians were allowed to add treatments while in the study if deemed necessary. Even though endoscopies were not performed, our data reveals that more patient treated with placebo/EcN reported blood in stools at the end of the study compared to all the other groups, which is an indicator of lack of mucosal healing in this group. A possible explanation for the negative effect of the placebo-EcN treatment, could be, that EcN in a symbiotic manner supports the possible pathogenic IBD-associated *E. coli* still present in these patients, who were not treated with Ciprofloxacin initially. EcN with regard to phylogenetic group and serotype is very similar to the IBD-associated *E. coli* pathotypes. A symbiotic support could be effectuated by nutrients made available by EcN for the IBD-associated *E. coli* or by increased biofilm formation involving both EcN and the IBD-associated *E. coli*. In support of such a mechanism, we have in a mouse colonization study found that IBD-associated *E. coli* strains reappeared after an initial Ciprofloxacin treatment, when mice were subsequently inoculated with EcN.[16]

In a study by Rembacken et al., EcN was found to be non-inferior (P = 0.0508) to mesalazine in induction of remission among patients with active UC, however, all their patients did also receive a one week induction with antibiotics. Furthermore, all patients were treated with topical or systemic prednisolone, thus making it impossible to truly evaluate the effect of EcN regarding induction of remission and impossible to compare this study to the one we present.[10] In a double blinded placebo controlled study of rectally administered EcN, it was found, that EcN enemas did improve remission rates in patients with left sided UC in a per-protocol analysis. However, it was found that no differences could be seen in an intention-to-treat analysis, since more patients in the groups treated with EcN were withdrawn from their study due to adverse events.[17] Furthermore, many patients in the study by Matthes et al. were excluded due to intake of non-permissible concomitant medication, making it difficult to compare these results with ours.[17]

Our study results could indicate that Ciprofloxacin does not benefit patients with UC, although, it wouldn't seem to worsen the outcome either. A short benefit in week 8 in patients receiving Ciprofloxacin alone could be due to the effect on the IBD associated microbiota, including the IBD-associated *E. coli*. It has previously been demonstrated in a meta-analysis that antibiotics do benefit patients with UC, the effect is however believed to be short lived,[18] and similarly, in our study mean CAI score, among patients treated with Ciprofloxacin alone, rose again in week 12.

Under the assumption that IBD-associated *E. coli* is part of UC pathogenicity, our study could be interpreted as if we simply did not find the right cure for these specific *E. coli*. Furthermore, our study results do, of course, not exclude that EcN could be beneficial and efficient in preventing relapses during remission of UC as shown in previous studies.[9,10,19] This effect could have been caused by the ability of a well-established EcN colonization to prevent re-infection with IBD-associated *E. coli*. It has been demonstrated that different *E. coli* strains, including colonization experiments with EcN, can co-exist based on the utilization of different nutrients.[20] However, it was also shown that infection with three different non-pathogenic *E. coli* including EcN were able to prevent re-colonization with a pathogenic (enterohemorhagic) *E. coli*.[20] Therefore, it is possible that EcN colonization of a non-inflamed human intestine, in the presence of other gram-negative bacteria, would be successful in preventing re-colonization with possible harmful bacteria such as the IBD associated *E. coli*.

We have used the CAI-score, which has proven efficient in determining flares of UC,[12] and if this symptom score occasionally will include patients without endoscopically active disease, this risk would have been the same in all four treatment groups in our study. Furthermore, all our patients had a well-established diagnosis of UC, including previous endoscopic evaluations. Finally, when looking specifically at the frequency with which patients reported blood in stools, probably reflecting mucosal healing, our results, regarding worse outcome of treatment with EcN without a prior antibiotic cure, were confirmed.

Our study provides an important lesson regarding the use of probiotics in general. Probiotics are not subjected to the comprehensive safety evaluation that pharmaceuticals receive, thus before recommending the use of probiotics more, or large scaled, randomized placebo-controlled studies are required. These concerns have become especially evident after one recent clinical trial studying probiotics in severe pancreatitis, found an unexpected increase in mortality in probiotic-treated patients.[21] Previously specific concerns regarding EcN has also been raised, after a case of sepsis with EcN in a preterm infant.[22] As with other forms of therapeutics, the safety and efficacy of probiotics should be considered on an individual (strain-by-strain) basis.

In conclusion, our data do not support the use of EcN as add-on therapy to conventional medication in acute flares of UC. Especially, EcN without a previous treatment with antibiotics, is, based on CAI-score, associated with fewer patients reaching clinical remission compared to conventional therapies. Our data, however, do not rule out that EcN is efficient in remission control, or that subgroups of patients with UC flares could benefit from EcN preceded by antibiotics.

Conflict of interest

The authors declare no conflict of interest.

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