ORIGINAL ARTICLE

Azithromycin and metronidazole versus metronidazole-based therapy for the induction of remission in mild to moderate paediatric Crohn's disease : a randomised controlled trial

Arie Levine,[1,2] Michal Kori,[3] Jarek Kierkus,[4] Rotem Sigall Boneh,[1] Malgorzata Sladek,[5] Johanna C Escher,[6] Eytan Wine,[7] Baruch Yerushalmi,[8,9] Jorge Amil Dias,[10] Ron Shaoul,[11] Gigi Veereman Wauters,[12] Mona Boaz,[13,14] Guila Abitbol,[15] Athos Bousvaros,[16] Dan Turner[17]

For numbered affiliations see end of article.

Correspondence to
Professor Arie Levine, Pediatric Gastroenterology and Nutrition Unit, Wolfson Medical Center, Holon 58100, Israel; alevine@wolfson.health.gov.il

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ABSTRACT

Objective Crohn's disease (CD) pathogenesis associated with dysbiosis and presence of pathobionts in the lumen, intracellular compartments and epithelial biofilms. Azithromycin is active in all three compartments. Our goal was to evaluate if azithromycin-based therapy can improve response and induce remission compared with metronidazole alone in paediatric CD.

Design This blinded randomised controlled trial allocated children 5–18 years with 10<Pediatric Crohn's Disease Activity Index (PCDAI)≤40 to azithromycin 7.5 mg/kg, 5 days/week for 4 weeks and 3 days/week for another 4 weeks with metronidazole 20 mg/kg/day (group 1) or metronidazole alone (group 2), daily for 8 weeks. Failures from group 2 were offered azithromycin as open label. The primary end point was response defined by a decrease in PCDAI>12.5 or remission using intention to treat analysis.

Results 73 patients (mean age 13.8±3.1 years) were enrolled, 35 to group 1 and 38 to group 2. Response and remission rates at week 8 were identical 23/35 (66%) in group 1 and 17/38 (45%) and 15/38 (39%) in group 2 (P=0.07 and P=0.025, respectively). The needed to treat for remission was 3.7. Faecal calprotectin declined significantly in group 1 (P=0.003) but not in group 2 (p=0.33), and was lower at week 8 (P=0.052). Additional therapy was required in 6/35(17%) from group 1 versus 16/38(42%) in group 2 (P=0.027) by week 8. Among 12 failures in group 2, open-label azithromycin led to remission in 10/12 (83%).

Conclusions The combination of azithromycin and metronidazole failed to improve response but was superior for induction of remission and reduction in calprotectin.

Trial registration number NCT01596894.

Significance of this study

What is already known on this subject?

- The importance of the microbiome in the pathogenesis of Crohn's disease (CD) suggests that strategies targeting the microbiota could improve patient care. The optimal antibiotic therapy to target both luminal and intracellular bacteria is unknown. Azithromycin is theoretically effective in controlling luminal and intracellular bacteria as well as biofilms, three compartments in which adherent invasive *Escherichia coli* or other pathobionts may reside. Rifaximin, azoles and quinolones do not target all three havens.

What are the new findings?

- The combination of azithromycin and metronidazole was superior to metronidazole alone for induction of remission though it did not reach superiority for response. It markedly reduced the need for additional therapy by week 8 compared with metronidazole alone and was associated with significant drop in C reactive protein and calprotectin.

How might it impact on clinical practice in the foreseeable future?

- Azithromycin-based therapy may be useful for induction of remission in children with active CD, reducing the need for immunosuppression agents such as steroids to induce remission in children.

INTRODUCTION

The important role of bacteria has long been recognised in the pathogenesis of Crohn's disease (CD). Evidence for the important role of gut bacteria includes identification of specific mucosal bacterial taxa that may adhere to and invade epithelial cells, presence of these bacteria in granulomas and the ability of certain *Escherichia coli* strains to replicate inside macrophage phagolysosomes.[1–8] Diversion of the faecal stream can lead to clinical improvement in medically refractory Crohn's colitis.[9 10] Disease susceptibility genes involved in CD are associated with innate immunity, recognition of bacterial pathogen and handling of intracellular bacteria.[11–14]

E. coli isolated from patients with CD may exist as biofilms, and creation of biofilms in turn may be associated with colonisation, adhesion and invasive

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properties[8 15] as well as antibiotic resistance.[16] Since these strains may exist in the lumen, adherent to the epithelium as a biofilm or as intracellular bacteria in macrophages, the ideal antibiotic would be effective in all three compartments. The probable role of bacteria in triggering disease activity could imply that antibiotics may have a role in managing flares and indeed several meta-analyses support the use of antibiotics.[17] However, the results of previous trials were heterogeneous in the intervention and methodology and many showed negative results.[18] A large fraction of the studies focused on the azole family of antibiotics (mainly metronidazole) and quinolones.[15 19–22] Eight of 13 studies, primarily with metronidazole and ciprofloxacin demonstrated no benefit for induction of remission.[16] Ciprofloxacin was shown to be superior to placebo for induction of remission in only one randomised controlled trial,[23] as has rifaximin.[24] Rifaximin is a non-absorbable antibiotic which would not be effective against bacteria that have already translocated in to mesenteric lymph nodes or replicating in macrophages. Ciprofloxacin is effective for intracellular bacteria but less so for bacteria in biofilms. Interestingly enough, clarithromycin, which is also a macrolide, was not better than placebo for induction of remission at 12 weeks.[25]

Unlike quinolones, rifaximin or azoles, which are active in only one or two compartments, azithromycin has excellent intracellular penetration, relatively high luminal concentrations and is effective against biofilms.[16 26]

Medications that can induce apoptosis are effective in treating CD (eg, thiopurines, biologics and thalidomide) given the defective apoptosis of activated T cells in CD.[27–29] Azithromycin is also a potent activator of apoptosis of T cells.[30–32] Thus, azithromycin could have added benefit in CD due to its antimicrobial properties or due to an immunomodulatory effect. Finally, azithromycin has been used in high doses for prolonged periods of time in cystic fibrosis with a good safety profile.[33–35]

We previously published our retrospective uncontrolled experience with a combination of metronidazole and azithromycin for active CD in children and adolescents over 8 weeks.[35] Clinical remission was observed in 21/32 (66%) of children and 54% of children with elevated C reactive protein (CRP) at baseline normalised their values.

The objective of the study was to evaluate the ability of azithromycin-based therapy to induce response and remission in children with active CD as an add-on to metronidazole in both groups. An add-on design was chosen since we did not feel that the use of placebo is ethical in children with active disease.[36]

METHODS

This was a 12-week investigator-initiated investigator-blinded randomised controlled trial involving two arms with 1:1 randomisation in children with mild to moderate active CD (National Institutes of Health NCT01596894). Patients were enrolled at 11 paediatric gastroenterology unit sites in six countries (Israel, Canada, the Netherlands, Portugal, Belgium and Poland) between 2012 and 2015. CD was defined following the revised Porto criteria.[37] Two investigators meetings were held prior to and during the study. This study was approved by the ethical committees of all participating sites and signed informed consent, and assent when required were obtained from all participants.

Intervention and eligibility

The treatment protocol was adapted from the previous report by Levine and Turner.[35] Eligible patients were randomised to one of two arms; group 1 received azithromycin (7.5 mg/kg to a maximum of 500 mg/once a day) for 5 consecutive days per week with a 2-day drug holiday for the first 4 weeks and then stepped down to 3 consecutive days per week of the same dose with a 4-day drug holiday over the subsequent 4 weeks. Both groups received metronidazole 20 mg/kg/day two times daily (maximum of 1000 mg/day) for 8 weeks (figure 1). A placebo design was not possible due to the regulatory hurdles involved in a multinational investigator initiated study.[36] Metronidazole was chosen as the control arm since it was the most commonly used antibiotic in paediatric CD at the time and to prove that the azithromycin component and not the metronidazole is responsible for the anticipated outcomes. Inclusion criteria were age 5–18 years, mild to moderate disease, as defined by the Pediatric Crohn's Disease Activity Index (PCDAI) >10 but ≤40 points,[38] at least one elevated inflammatory marker above normal values (ie, CRP, erythrocyte sedimentation rate (ESR) or calprotectin) and disease duration since diagnosis <3 years. Patients were excluded if they had a stool pathogen (culture, parasites or *Clostridium difficile* toxin) involvement of the proximal ileum or jejunum (L4b as per Paris classification) which determined disease location,[39] IBD unclassified, presence of fibrostenotic disease (defined as strictures with prestenotic dilatation), internal or perianal fistulising disease, prominent extraintestinal manifestations (eg, arthritis, uveitis and sclerosing cholangitis), known allergy to either metronidazole or azithromycin, prolonged QTc at baseline ECG or steroid use during the 7-day preceding enrolment.

Patients on immunomodulators were allowed to continue therapy without a dose change if stable for 8 weeks prior to enrolment. Physicians had the option of adding a thiopurines from week 4, as thiopurines are not expected to alter disease activity within the 4 weeks remaining to the end of the trial and the ethical committees raised concerns about withholding immunomodulators for 8 weeks after induction of remission. Introduction of any other medication for treatment of CD after enrolment and prior to 8 weeks was considered treatment failure on the intention-to-treat (ITT) principle.

Study procedures

Patients were seen and examined in clinic at weeks 0, 4, 8 and 12 and had an additional telephone visit at 48 hours after start of study to ensure tolerance, safety and compliance. The week 12 visit was added to evaluate changes in therapy, including patients who received open-label azithromycin from week 8, as well as adverse events resolution.

Patients who were intolerant to the medications at the 48-hour telephone visit (predefined as new onset of emesis, significant nausea, abdominal pain or diarrhoea) were instructed to reduce the dose of metronidazole by 25% to 15 mg/kg/day and divide the frequency to three times daily. In a modified ITT principal, patients who withdrew during the first 48 hours were excluded from the study.

At screening, children were tested for stool pathogens and underwent an ECG with measurement of QTc. Each visit included review of adverse events, physical examination, anthropometry measurement, determination of disease activity by a Physicians Global Assessment (PGA) including seven possible measures of activity ranging from normal, not ill to most extremely ill as well as PCDAI and wPCDAI.[40] CRP, ESR, a complete blood count and albumin were also recorded at each visit. Faecal calprotectin was measured at week 0 and 8. Stool samples were frozen on site at −20°C and shipped to a central laboratory at the end of the study. Stool extracts were obtained using BUHLMANN Smart-prep kit. Faecal calprotectin was measured using the fCAL

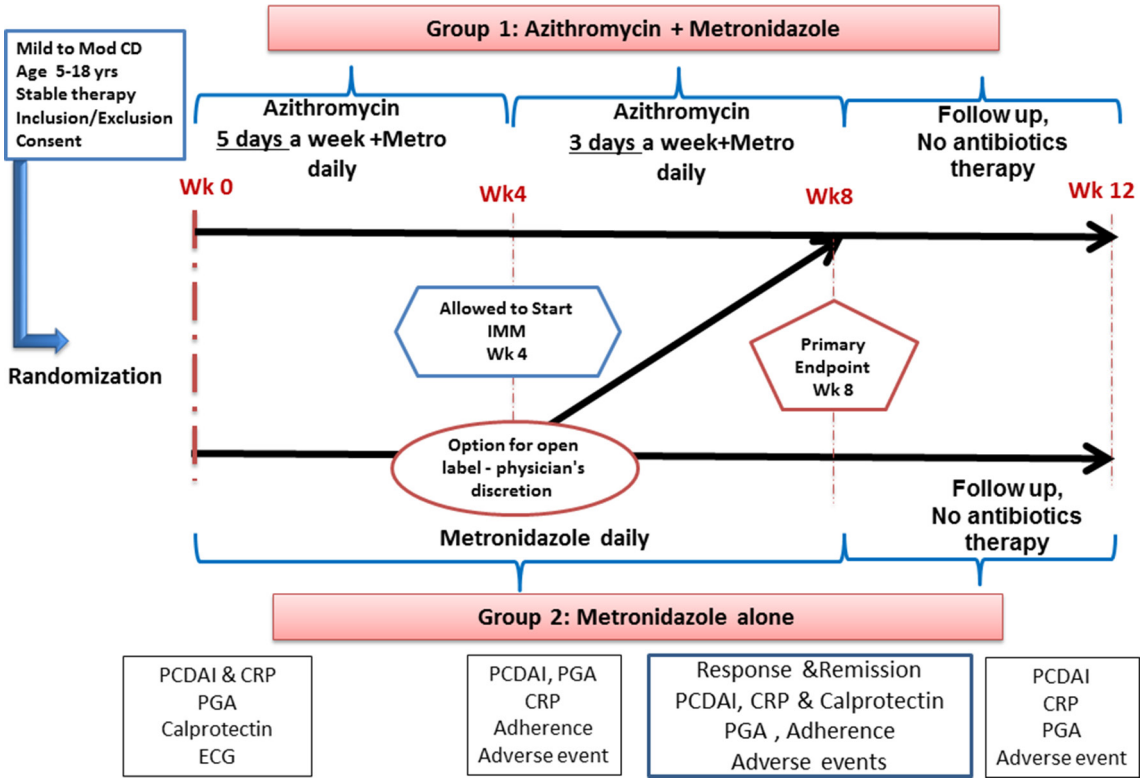

Figure 1 Study design Azithromycin for Crohn (AZCRO) trial. CD, Crohn's disease; CRP, C reactive protein; IMM, immunomodulators; PCDAI, Pediatric Crohn's Disease Activity Index ; Mod, moderate; PGA, Physicians Global Assessment; Wk, week; yrs, years.

ELISA kit (Buhlmann AG Labs, Switzerland) with a normal range <100 µg/g. Preliminary results indicated very high levels of faecal calprotectin (>1800 µg/g) in many patients; therefore, all samples were diluted 1:10 according to manufacturer's instructions.

Patients determined to be refractory at any time point or who deteriorated were treated according to the physician's discretion; these patients were labelled as failures in the ITT analysis with non-response imputed. The week 8 visit was scheduled several days after cessation of the antibiotics to prevent a biased PCDAI due to antibiotic-associated diarrhoea. Antibiotic-associated diarrhoea was defined as diarrhoea that resolved within days of ceasing antibiotics.

Children in group 2 who failed metronidazole (no improvement or deterioration) between week 4 and 8 could be offered use of azithromycin as in group 1 in an open-label fashion with a 4-week follow-up visit and were analysed separately.

Outcomes

The primary outcome was a good response defined as a drop in PCDAI of ≥12.5 points or remission (PCDAI≤10) at week 8, since patients with mild disease were enrolled and could be in remission with a drop of <12.5 points from baseline.

The secondary outcomes were clinical remission (ie, PCDAI≤10) at week 8, normalisation of CRP (ie, CRP≤0.5 mg/dL) and mean faecal calprotectin level at week 8. We also evaluated adverse events, need for treatment escalation to induce remission (including steroids, other antibiotics, exclusive enteral nutrition or biologics) and compliance captured by the Medication Adherent Rating Scale (MARS) adherence questionnaire completed at each visit.[41] Good compliance was defined as full compliance or if patients missed only an occasional dose (≤1 dose a week).

Patients who failed to respond, required additional therapy or withdrew because of side effects were considered failures.

Randomisation and concealment of allocation

Patients were randomised 1:1 in previously generated random blocks of 6 by sealed, numbered and opaque envelopes. Envelopes had to be used consecutively and opened only after informed consent was obtained. The codes were held by the study project manager in a sealed file at the Wolfson Medical Center paediatric inflammatory bowel disease (PIBD) research unit and were not available to any of the participating sites or physicians. Each site received a sealed envelope with the site's treatment allocations and they were instructed to open only in case of emergency but none eventually occurred. To maintain blinding of the treating physician, patients received instructions for both medications. The envelope, with precise allocation instructions, only was opened after leaving the physician's office with a research coordinator or nurse. Participants were asked not to discuss treatment allocation with their physician, but questions were referred to another uninvolved physician. This ensured concealment of allocation.

Sample size calculation

Based on the results of our previous study,[35] we assumed that the response rate would be 65% and 30% in the combination versus metronidazole arms, respectively. Under these assumptions, we needed to study 31 subjects in each group to be able to reject the null hypothesis with probability (power) 0.8 and type I error of 0.05 using an uncorrected χ^2. Assuming 10% dropout rate and 5% possible protocol violations rendering patients ineligible, we aimed to enrol 72 patients.

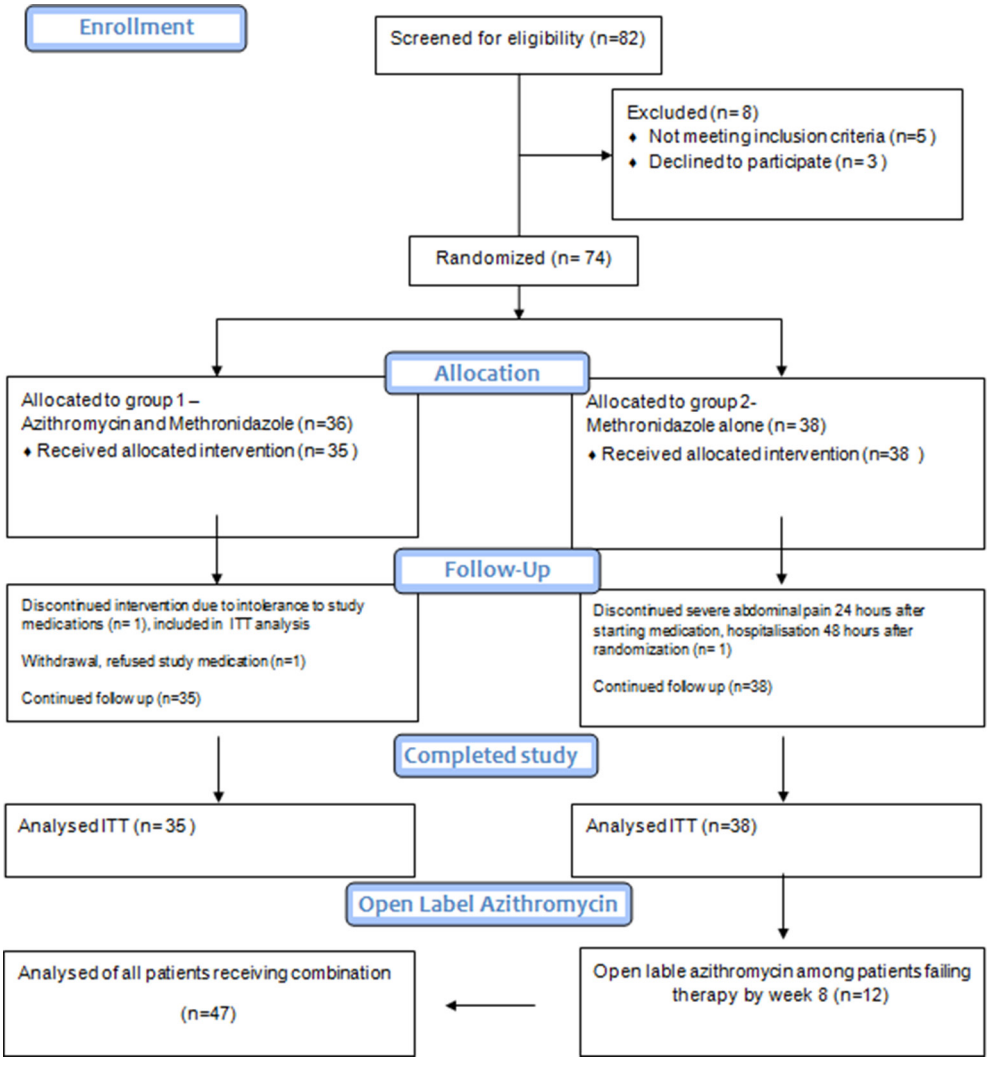

Figure 2 Consort diagram: flow of patients in the study. ITT, intention to treat.

Statistical analysis

Data were queried and entered twice by two different coordinators, and discrepancies resolved by referring to the source document. Continuous variables with normal distribution are presented as mean±SD. Variables with non-normal distribution are presented as median with IQR. Within group differences before and after treatment (eg, biochemistry measures) were explored using the paired Student's t-test. Nominal data were summarised as frequencies and rates and compared using the χ^2 or exact tests, as appropriate. We used the modified ITT principal in which patients were included if they had taken the study medications for at least 48 hours. Data of subjects failing treatment or lost to follow-up were carried forward for the ITT analysis with non-response imputed for response and remission and last observation carried forward for continuous variables. All tests were two sided and considered significant at $P<0.05$. Data were analysed using SPSS V.22 software (IBM).

RESULTS

Eighty-two patients were screened and 74 randomised, of whom 73 took the study medication and were eligible for participation (figure 2). Three patients reported intolerance (nausea or abdominal pain) during the first 48 hours of treatment, resolving following dose reduction in two and spontaneously in the third; therefore, no patient was withdrawn during that initial period. There were no differences at baseline between groups for any of measures of severity or use of immunomodulators (table 1). However, the groups differed by gender with group 1 having more female patients and a trend for more colonic involvement (L2+L3).

Response to therapy

Comparison of response, remission, normal CRP rates and need for additional therapy by week 8 are portrayed in figure 3. Response, the primary outcome, was numerically superior in the combination group versus the metronidazole group, but this did not reach statistical significance ($P=0.07$). This was due to only two patients in the metronidazole group with moderate disease who achieved response but did not obtain remission. In contrast, remission rates were significantly higher in the combination group, while the need for treatment escalation by week 8 was significantly lower ($P=0.025$; figure 3). All patients in remission on their assigned treatment at week 8 were in corticosteroids (CS)-free remission by ITT. The number needed to treat (NTT) for remission was 3.7.

Median calprotectin at week 8 was 1365 (737–2867) µg/g in the azithromycin group compared with 2679 (1203–4660) µg/g in the metronidazole group ($P=0.052$). Median calprotectin

Table 1 Demographic data

Baseline	Group 1 azithromycin+metronidazole (n=35)	Group 2 metronidazole (n=38)	Total cohort (n=73)
Male	8 (22.9%)	20 (52.6%)	28 (38.4%)**
Age (years)	13.9±3.2	13.6±3	13.8±3.1
Range age onset	6–18.1	8.1–18.3	6–18.3
Disease duration (years) (median (IQR))	0.83 (0.16–1.6)	0.37 (0–1.1)	0.5 (0–1.3)
New onset disease	9 (25%)	18 (47%)	27 (36%)
Location (Paris classification)			
L1	6 (17.1%)	14 (36.8%)	20 (27.4%)
L2	3 (8.5%)	1 (2.6%)	4 (5.4%)
L3	26 (74.2%)	23 (60.5%)	49 (67.1%)
L4	19 (54.3%)	18 (47.4%)	37 (50.7%)
Colonic involvement (either L2 or L3)	29 (82.9%)*	24 (63.2%)	53 (72.6%)
Ileal involvement (either L1 or L3)	32 (91.4%)	37 (97.4%)	69 (94.5%)
PCDAI			
Baseline PCDAI (median (IQR))	20 (15–30)	17.5 (12.5–27.5)	17.5±8.7
Mild (10–27.5)	25 (71%)	33 (86%)	58 (79%)
Moderate (30– 40)	10 (28%)	5 (13%)	15 (20%)
Baseline CRP (mg/dL (normal<0.5)	2.6±2.3	2.9±2.4	2.8±2.4
Baseline calprotectin (µg/g) (median (IQR)) (normal<100)	2830 (1523–4465)	3130 (2150–3130)	2920 (1871.25–4415)
Baseline ESR (normal<20)	34.4±20.6	34.1±15.1	33.9±17.8
Baseline albumin (g/L)	3.9±0.5	3.8±0.4	3.9±0.4
Baseline IMM (%)	17 (48%)	13 (34%)	30 (41%)

* Trend for colonic involvement P = 0.06.
Only gender differed significantly **P<0.01.
CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IMM, immunomodulators; PCDAI, Paediatric Crohn's Disease Activity Index.

declined significantly in the azithromycin group from 2815 (1609–4112) µg/g to 1365 (737–2867) µg/g, P=0.001, while in the metronidazole group the decline was not significant, 3040 µg/g (2185–4415) to 2679 µg/g (1203–4660), P=0.163.

Other changes by groups are portrayed in figure 4 and figure 5. Median PCDAI decreased from 20 (15-30) to 8.75 (2.5–15.6) after 8 weeks, P<0.0001 in the azithromycin group and decreased from 17.5 (12.5–27.5) 12.5 (5–26.8), P<0.0001 in the metronidazole group. wPCDAI decreased from 32.5 (27.5–52.5) to 15 (7.5–30), P<0.0001 in the azithromycin group and decreased from 31.2 (24.3–40.6) to 20 (7.5–35.6), P=0.009 in the metronidazole group. CRP decreased from 1.6

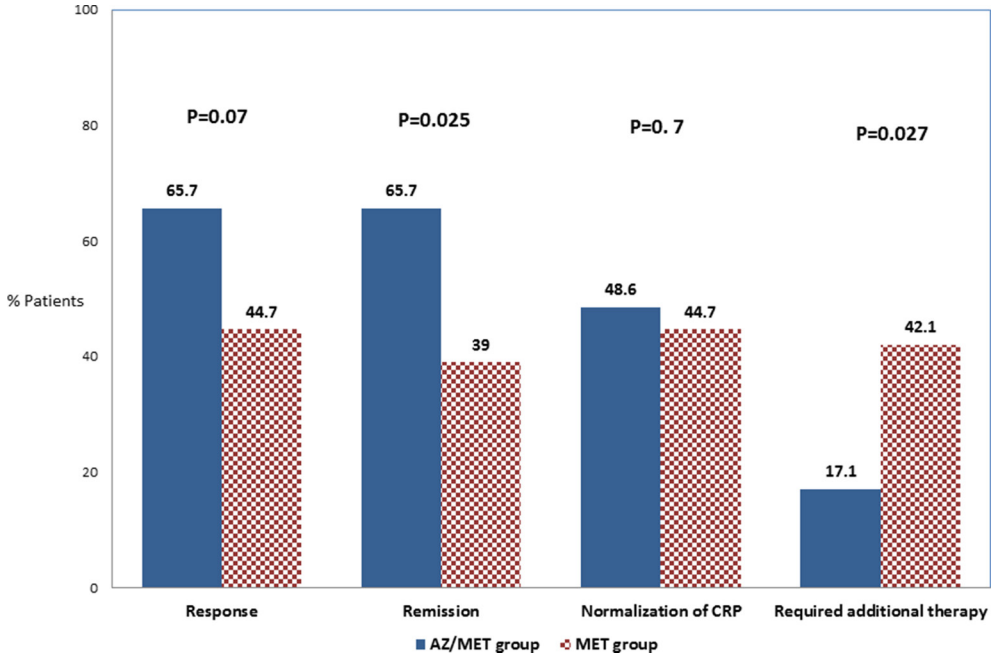

Figure 3 Comparison of clinical outcomes between treatment groups at week 8. AZ/MET, azithromycin/metronidazole; CRP, C reactive protein.

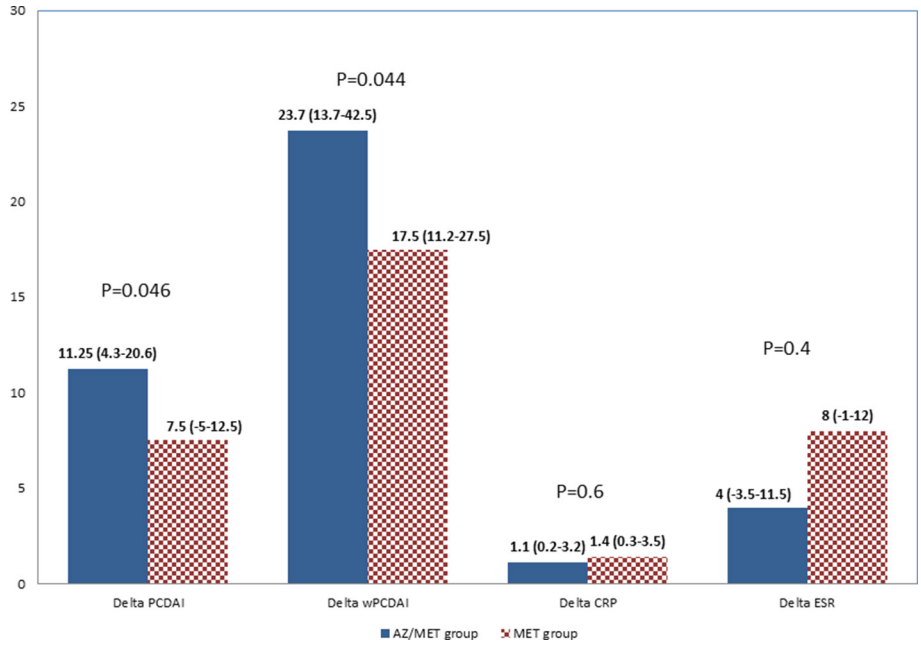

Figure 4 Changes in median clinical and inflammatory parameters within groups by week 8. Values are presented as delta of the median (IQR) between week 0 and week 8. CRP, C reactive protein; ESR, erythrocyte sedimentation rate; PCDAI, Pediatric Crohn's Disease Activity Index .

(0.6–4) to 0.5 (0.2–0.8), P<0.0001 in the azithromycin group, whereas in the metronidazole CRP dropped from 2.1 (1.1–4.6) to 0.6 (0.3–1.5), P<0.0001. In addition, ESR decreased from 30.5 (16.2–52.7) to 27 (12.7–45), P=0.078 in the azithromycin group and decreased from 34 (23-44) to 25 (14.2–42), P=0.005 in the metronidazole group. There was no difference between groups for ESR at week 8.

Thiopurines could be started in immunomodulator-naive patients per protocol at week 4, and this was the case in 6/35 (17.1%) of patients in the azithromycin group and 9/38 (23%) patients in the metronidazole group. Among patients receiving azithromycin, 3/6 (50%) were in remission at week 8, whereas only 3/9 (33%) in the metronidazole group were in remission at week 8.

There were however significant differences with regard to need to change therapy to induce remission (aside from adding thiopurines), portrayed in figure 3, as many more patients in the metronidazole group (42%) required additional therapy for induction of remission by week 8. Among patients' failing therapy in the metronidazole group, 12/22 (50%) were switched to receive open-label therapy with azithromycin added to their

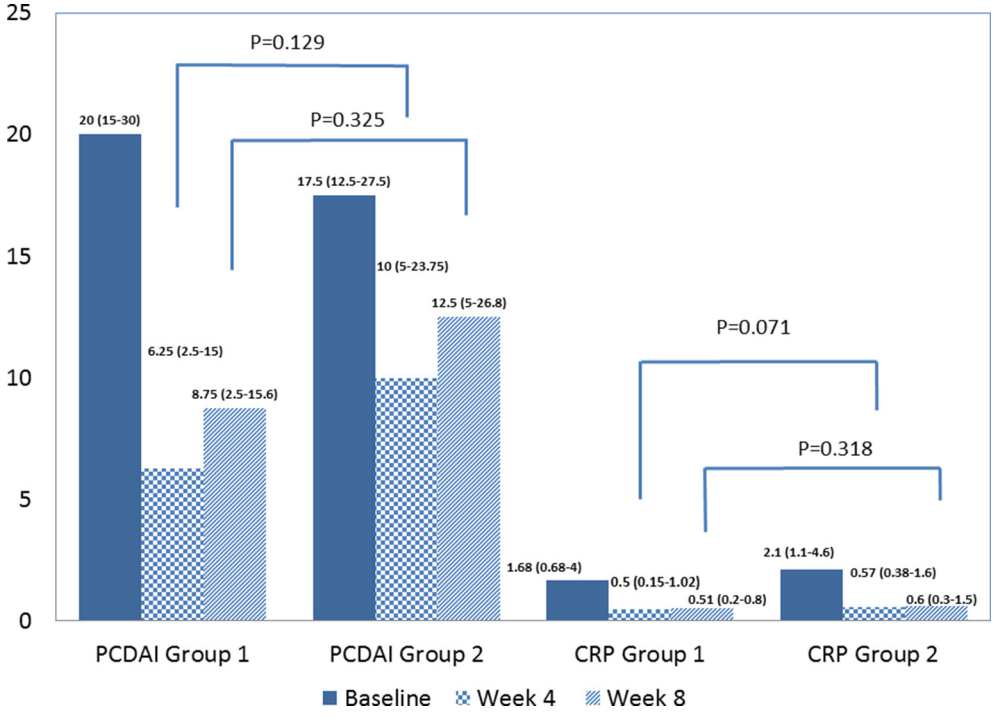

Figure 5 Changes in disease activity and CRP over time. Values are presented as median (IQR). CRP, C reactive protein; PCDAI, Pediatric Crohn's Disease Activity Index.

Table 2 Predictors of response to antibiotic therapy

Baseline	Remission (n=40)	No remission (n=33)	Total cohort (n=73)	P value
Age (years) (mean±SD)	13.2±3.6	14.5±2.2	13.8±3.1	0.05
Female	29 (72%)	15(45.4%)	44	0.03
Location (Paris classification)				
L1	9 (47.4%)	10 (52.6%)	20 (27.4%)	0.76
L2	2 (66.7%)	1 (33.3%)	4 (5.4%)	
L3	27 (55.1%)	22 (44.9%)	49 (67.1%)	
L4	23 (65.7%)	12 (34.3%)	37 (50.7%)	0.04
Colonic involvement (either L2 or L3 and not L1)	29 (55.8%)	23 (44.2%)	53 (72.6%)	0.5
Ileal involvement (either L1 or L3 and not L2)	36 (52.9%)	32 (47.1%)	69 (94.5%)	0.5
Baseline PCDAI median (IQR)	16.25 (15–23.13)	22.5 (13.75–30)	20.6±8.5	0.113
Baseline CRP (mg/dL) median (IQR)	1.7 (0.7–3.5)	2.7 (1.4–5.6)	2 (0.9–4.2)	0.06
Baseline calprotectin (µg/g) median (IQR)	2485 (1864–3220)	3415 (1984–5630)	2890 (1967–4304)	0.06

CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IMM, immunomodulators; PCDAI, Paediatric Crohn's Disease Activity Index.

previous metronidazole. Within 4 weeks, clinical remission was obtained in 10/12 (83%) of these patients.

Predictors of response to therapy

Predictors of response to antibiotic therapy are presented in table 2. Younger children, gender and those with L4 disease responded better, while patients with lower inflammatory burden trended to respond better to therapy, though the latter were not significant. We did not analyse predictors within groups as the numbers were too small to be meaningful.

Compliance to therapy

Compliance was assessed by the MARS questionnaire and direct count; 28 (85%) reported high compliance in the azithromycin group versus 34 (92%) in the metronidazole group (P>0.1).

Adverse events

There were 18 adverse events reported on 13 patients in the azithromycin group (37%) and 29 adverse events involving 17 patients in the metronidazole group (44%), as shown in table 3. Notably, three patients in the first group developed transient mild alanine aminotransferase elevation; this led to discontinuation in two. One patient in group one was withdrawn due to suspected metronidazole induced paraesthesia, though this was subsequently found to be unrelated to therapy and resolved spontaneously.

Only one severe adverse event (SAE) was reported, from the metronidazole group (hospitalisations due to worsening disease). There were no drug-related SAEs in the study.

DISCUSSION

In this single-blinded randomised controlled trial, we failed to demonstrate superior response rates (the primary end point) for patients receiving azithromycin-based therapy though patients receiving the combination with azithromycin had a trend towards higher response rates (66% vs 45%, P=0.07) and achieved significantly better remission. There were twice as many patients with moderate (as opposed to mild disease) in group 1, and despite this remission and response were better in group 1.

On the other hand, remission rates with azithromycin and metronidazole were significantly higher than with metronidazole alone, with a 66% clinical remission and an NTT of 3.7. This was also supported by the fact that significantly more patients in the metronidazole arm required additional therapy by the treating physician who was blinded to the treatment allocation. The open-label data from this study also suggest that most of those failing metronidazole alone entered remission after the addition of azithromycin. Both antibiotic regimens caused a drop in CRP. Though the decline in CRP was similar in both groups, calprotectin declined significantly only in the group treated with azithromycin. There was no significant difference in adverse or SAEs between groups.

Table 3 Adverse events (AEs)

Patients, n=30; AEs=47	Azithromycin, n=13; AE=18	Discontinued therapy, n=5	Metronidazole, n=17; AE=29	Discontinued therapy, n=1
Transient ALT elevation	3	2	0	
Abdominal pain	2		5	1
Diarrhoea	3	1	5	
Dizziness	1	1	0	
Nausea	1		1	
Vomiting	2		4	
Herpes zoster	1		0	
Paraesthesia	1	1	0	
Candida thrush	0		1	
Syncope	0		1	
Fever	2		1	
Rash	0		1	
Backache	0		1	
Rhinitis	1		0	
Vulvitis	0		1	
Glossitis	0		2	
Black tongue	0		1	
Headache	0		2	
Rectal bleeding	0		1	
Weakness	0		1	
Influenza-like symptoms	0		1	
Other undefined	1		0	
Cardiac arrhythmias	0		0	

ALT, alanine transaminase.

Another unusual observation from our study was almost no difference in remission and response rates within each treatment. This 'all or none' phenomenon suggests that the effect of antibiotics may depend on the type of the microbiota involved and on its susceptibility to antibiotics, such that patients with resistant bacteria either go in to remission or do not respond, acknowledging that even within the microbiota, the individual effect vary between patients.

The relatively high response and remission rates in the metronidazole group was surprising in light of several studies in adults demonstrating that remission rates with metronidazole were not superior to placebo.[42 43] However, our study differed by the fact that it was performed in children with very short disease duration, a cohort that might be more responsive to medical therapy, especially antibiotics.[44] An additional difference, typical of a paediatric cohort of patients with CD, is that the majority of children had colonic involvement which might respond better to antibiotic therapy.[20] There were significantly more female patients and a trend towards more colonic disease in the azithromycin group. Additional predictors of response to therapy were younger age and lower inflammatory burden, suggesting that this therapy may be more beneficial for children with milder inflammation.

Azithromycin has several intriguing properties that make it an attractive candidate for inducing remission.[30–35 45 46] Remission rates found here replicate the rates reported in the earlier retrospective study.[35] This therapeutic approach may allow expanding options that do not involve immune suppression.

Data regarding specific targeting of adherent-invasive *E. coli* (AIEC) are sparse. In vitro studies have been found colicins to be effective against AIEC isolates even when present as a biofilm. Ciprofloxacillin and colicins were both effective against AIEC-infected macrophages though ciprofloxacillin had superior intracellular activity.[47] These results may suggest that alternative combinations such as azithromycin with ciprofloxacillin or colicins and ciprofloxacillin may be more effective for AIEC.

To our knowledge, this is the first blinded randomised controlled trial of any antibiotics in paediatric CD. A strength of the study was its multinational single-blinded design, which indicates that this can be translated across geographical areas, though differences in antibiotic sensitivities may differ and may change over time with increased use. A shortcoming of this investigator-initiated trial is that mucosal healing was not investigated. However, this was only an 8-week intervention, and objective markers of inflammation were obtained before and after therapy. CRP decreased significantly from baseline after treatment in both groups and the differences in calprotectin at week 8 bordered on significance and decreased significantly only in the azithromycin group.

Our data contribute to the growing body of evidence, suggesting that targeting the microbiota may be useful as a therapeutic strategy for induction of remission in children with mild to moderate disease. There remain many unanswered questions regarding antibiotic therapy at the present time. Our study design did not allow us to study the effect of azithromycin monotherapy. The trial design was based on our previous success with the combination of azithromycin and metronidazole, which we replicated in the current study with identical remission rates. We chose a 5-day/week protocol with 2-day washout to prevent systemic accumulation of azithromycin, which is a theoretical concern given the reports of QT prolongation with azithromycin in elderly adults.[48] However, this risk appears to depend on age and prior cardiovascular risk.[49] In a prospective observational study of 47 individuals with low cardiovascular risk, ECGs were performed before and after 5 days of azithromycin treatment, there was no significant increase in QTc. A cohort study involving patients without cardiovascular disease failed to find an association between use of azithromycin and significant QTc prolongation.[49 50]

Although the primary outcome of this trial was missed by a marginal statistical significance, the consistent results from all other outcomes lead us to conclude that azithromycin-based therapy may be a viable option in mild to moderate paediatric CD.

Author affiliations
[1]Pediatric Gastroenterology and Nutrition Unit, Wolfson Medical Center, Holon, Israel
[2]Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israel
[3]Pediatric Day Care Unit, Kaplan Medical Center, Rehovot, Israel
[4]Department of Gastroenterology, Hepatology, Feeding Disorders and Pediatrics, The Children's Memorial Health Institute, Warsaw, Poland
[5]Department of Pediatrics, Gastroenterology and Nutrition, Jagiellonian University Medical College, Cracow, Poland
[6]Department of Pediatric Gastroenterology, Erasmus MC-Sophia Children's Hospital, Rotterdam, The Netherlands
[7]Division of Pediatric Gastroenterology, Department of Pediatrics, University of Alberta, Edmonton, Canada
[8]Pediatric Gastroenterology Unit, Soroka University Medical Center, Beersheba, Israel
[9]Faculty of Health Sciences, Ben-Gurion University of the Negev, Negev, Israel
[10]Department of Pediatrics, S. Joao Hospital, Porto, Portugal
[11]Pediatric Gastroenterology Unit, Ruth Children's Hospital, Rambam Medical Center, Brussels, Belgium
[12]Pediatric Gastroenterology Unit, University Hospital UZ Brussels, Brussels, Belgium
[13]Department of Nutrition School of Health Sciences, Ariel University, Ariel, Israel
[14]Epidemiology and Research Unit, E. Wolfson Medical Center, Holon, Israel
[15]Pediatric Gastroenterology Lab, The Juliet Keidan Institute of Paediatric Gastroenterology, Hepatology, and Nutrition, Shaare Zedek Medical Center, Jerusalem, Israel
[16]Division of Gastroenterology and Nutrition, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts, USA
[17]The Juliet Keidan Institute of Pediatric Gastroenterology and Nutrition, Shaare Zedek Medical Center, The Hebrew University of Jerusalem, Jerusalem, Israel

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