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CLINICAL TRIAL REPORT

Pilot Study of IL-1 Antagonist Anakinra for Treatment of Endometriosis

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Purpose: To evaluate the impact of an interleukin-1 (IL-1) antagonist anakinra (Kineret®) on endometriosis-related quality of life (QoL), pain, and inflammatory biomarkers.

Methods: This was a single-site, randomized, double-blinded, placebo-controlled, cross-over pilot clinical study of patients recruited at an academic specialty clinic. Eligible participants were females aged 18–45 years with menstrual cycles every 24–32 days. Subjects had moderate to severe dysmenorrhea and either a surgical diagnosis of endometriosis or an endometrioma on imaging. Subjects were randomly assigned in a double-blind fashion to receive either the study drug or placebo administered as daily injections during the first 3 periods and then the alternate intervention for the next 3 periods.

Results: Fifteen subjects completed the 6 menstrual cycle study. After each period, they completed the Endometriosis Health Profile-30 (EHP-30) QoL questionnaire and an assessment of dysmenorrhea using a 0–100 Visual Analogue Scale (VAS). All domains of the EHP-30 showed a trend towards improvement, with significant improvements in powerlessness (54.5 vs 63.3, $p = 0.04$) and self-image (58.1 vs 66.7, $p = 0.03$) on the study drug compared to placebo. The mean dysmenorrhea VAS also trended toward improvement with a score of 37.5 during active treatment and 42.6 with placebo ($p = 0.26$). No difference in menstrual cycle length was detected (29.3 days vs 27.7 days, $p = 0.56$). There were significant differences in multiple inflammatory biomarkers between the study drug and placebo, including BDNF, IL-1, and IL-6 among certain groups.

Conclusion: With all EHP-30 domains and the dysmenorrhea VAS showing either a statistical improvement or trend towards improvement, there is justification for a larger study. As no impact on menstrual cycles was detected, anakinra may be a particularly impactful option for women desiring fertility. Additional evaluation is needed on the role of anakinra on inflammatory markers given significant reductions were identified in multiple biomarkers.

Plain Language Summary: Endometriosis is a common gynecologic disease afflicting millions of patients. Anakinra is an IL-1 antagonist currently used for treatment of rheumatoid arthritis which has been found to improve quality of life measures for patients with endometriosis. Anakinra also reduces levels of biomarkers known to be associated with endometriosis-related inflammation. More study is needed on the role of anakinra in improving endometriosis symptoms.

Keywords: endometriosis, IL-1 antagonist, anakinra, Kineret, pelvic pain, quality of life, inflammation

Introduction

Although 5–10% of reproductive aged women is the generally accepted prevalence for endometriosis, there is also broad acceptance that endometriosis is likely underdiagnosed.[1] At its core, endometriosis is an estrogen-dependent, progesterone-resistant, chronic inflammatory disease that inflicts infertility and pelvic pain. As a result, endometriosis is associated with notable decreased quality of life in the women who suffer from this disease, with impacts on both physical and mental health.[2,3] Yet, despite best efforts and introduction of novel hormonal and non-hormonal therapeutics over the

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past decade, treatment options remain sub-optimal.[4,5] Current medical therapies to treat the pain symptoms of endometriosis are often of limited benefit and temporary in nature, and unfortunately produce significant side effects which can limit their tolerability and ultimately their use.[6,7] All current US Food and Drug Administration (FDA) approved medications including GnRH agonists and antagonists, danazol, and Depo-Provera prevent or contradict pregnancy, which places women who are concurrently trying to conceive into a position with limited options.

There are multiple theories about the etiology of endometriosis. Inflammation is a well-established central theme in the pathophysiology of endometriosis.[8,9] One leading mechanism involves a positive feedback cycle in which estrogen drives increased activity of COX2 and PGE2 leading to increased inflammation, which itself leads to increased activity of aromatase, thereby producing estrogen.[10–12] Research is ongoing into immune checkpoint molecules, such as cytotoxic T lymphocyte-associated antigen-4 (CTLA4), which may have a role in maintaining chronic peritoneal inflammation.[13] Beyond driving pain, the inflammatory response also promotes progesterone resistance, limiting the efficacy of many of the most common medical therapies.[14,15] In this model of inflammation-driven pain, interleukin-1 (IL-1) is a key inflammatory mediator previously linked in the pathophysiology of endometriosis.[16–18] IL-1 itself is central to the inflammatory profile throughout the body and is released by macrophages which are a predominant inflammatory cell type found in the presence of endometriosis lesions.[19] Data is increasingly suggesting that IL-1 is a mediator of inflammation in endometriosis.[20,21] A recent meta-analysis evaluating multiple interleukins identified that IL-1RA (receptor antagonist) levels were 2.56 standard deviations higher in those with endometriosis compared to those without.[22] Despite multiple studies connecting IL-1 and endometriosis, the potential therapeutic benefit of IL-1 antagonism to reduce inflammation and therefore reduce symptoms such as endometriosis-related pain remains unexplored.[14]

Anakinra (Kineret®, Swedish Orphan Biovitrum) is an IL-1 antagonist currently FDA- approved for the treatment of rheumatoid arthritis. Given the involvement of IL-1 in the pathophysiology of endometriosis, anakinra may be a possible therapy for this disease, including for women seeking fertility. Anakinra has been found to reduce signs and symptoms of rheumatoid arthritis while being safe and well tolerated.[23] The medication has also been considered or utilized for treatment of multiple other inflammatory-based diseases including amyloidosis, pericarditis, asthma, Kawasaki disease, multi-system inflammatory syndrome, and COVID-19 with promising results.[24–26] Prior evaluation of anakinra in diabetic rat models found decreased endothelial dysfunction (which is associated with IL-1), less oxidative stress, and less inflammation.[27,28] Importantly, anakinra, at significantly higher doses than prescribed to humans, does not adversely affect fertility or fetal outcomes in animal studies.[29] We postulate that anakinra administration will decrease subjective pain scores and suppress key endometriosis associated inflammatory markers in women suffering from endometriosis without adverse effects on menstrual cycle characteristics. This study is unique in that it is the first to investigate the impact of an IL-1 antagonist on endometriosis and is a non-hormonal medication that could be compatible with pregnancy.

Materials and Methods

Study Description and Patient Recruitment

This was a single-site, randomized, double-blinded, placebo-controlled, cross-over pilot clinical trial at the University of California at San Diego (UCSD) evaluating use of anakinra for the treatment of endometriosis-related pelvic pain and quality of life. Swedish Orphan Biovitrum (SOBI) sponsored this investigator-designed study and provided the study drug and placebo. The study was approved by the Institutional Review Board at UCSD and registered on ClinicalTrials.gov (NCT03991520). The study complies with the Declaration of Helsinki.

Study participants were recruited from the UC San Diego Center for Endometriosis Research and Treatment if they had dysmenorrhea thought to be secondary to endometriosis. Eligible participants were females aged 18–45 years with regular painful menstrual cycles every 24–32 days and menstrual periods lasting no more than 10 days. All participants had surgically proven endometriosis or imaging findings of endometrioma within the 5 years prior to enrollment. They had a moderate to severe dysmenorrhea score of at least 2/3 using the Biberoglu & Behrman (B&B) scale question. The B&B scale questions are graded on a scale of 0 to 3, with higher numbers indicating more severe symptoms. Exclusion criteria included history of hysterectomy or oophorectomy, current pregnancy, attempting pregnancy, plan to receive

a live vaccine during the study, or other contraindication to anakinra. Subjects with a history of non-response to GnRH agonist/antagonist, Depo-Provera, danazol or letrozole were also excluded as we did not want to include patients whose dysmenorrhea may be due to reasons other than endometriosis. Other exclusion criteria included clinically abnormal baseline labs, including blood count and liver and kidney function tests. The planned sample size for this pilot study was 20, consistent with other early-stage exploratory studies.[30,31]

Prior to voluntary study enrollment, participants were provided with basic information about the study by the physician and then referred to a research nurse for consent if they expressed interest. Non-investigational alternatives to anakinra were offered, including medical therapy and surgery. Subjects on hormonal therapy were instructed not to change their regimen for the duration of the study. Those that had recently stopped contraceptives had a one month wash out period and those who had recently stopped FDA-approved endometriosis medications had a three month wash out period. Long wash out periods were chosen to essentially eliminate the chance of any continuing impact of the prior medication influencing the study period. Participants received $100 at the end of each menstrual cycle, for a total of $600 possible compensation.

Once patients were consented and confirmed to meet entry criteria for the study, they were randomly assigned using block randomization to one of two groups. Treatment Group A received 100 mg anakinra daily during their first three periods and then placebo daily during the next three periods. Treatment Group B received placebo for the first three periods and then 100mg anakinra daily for the next three periods. Randomization was conducted and interventions dispensed by the UCSD investigational pharmacy. Study participants and study staff were blinded to group assignment. The study was unblinded after all treatments were completed and data collected.

Both anakinra and placebo were self-administered as daily subcutaneous injections taken during menses. Participants were provided with all materials required for safe injection and disposal of needles at home. Administration was started within twenty-four hours of the first day of menstrual flow and continued daily until within twenty-four hours of the last menstrual day. The 100 mg dose of anakinra is the standard FDA-approved dose for rheumatoid arthritis and was therefore utilized in this pilot study.

At the first visit with the research nurse and after each period, participants filled out 3 separate questionnaires. Multiple questionnaires were utilized given the wide variation in symptomatology:

A. Pelvic pain was assessed using the dysmenorrhea component of the widely used B&B score: 0 Absent (no discomfort), 1 Mild (some loss of work), 2 Moderate (In bed part of one day, occasional loss of work), 3 Severe (In bed one or more days, incapacitation). The full B&B score also includes assessment of pelvic pain and dyspareunia as well as exam findings of tenderness and induration.[32] However, we chose to utilize the dysmenorrhea component only given our focus was on the impact of anakinra on dysmenorrhea specifically.
B. Pelvic pain was also assessed using a visual-analog scale (VAS) for dysmenorrhea daily during each of the six studied menses. It is scaled from zero to one hundred with zero indicating no pain and one hundred reflecting the worst pain. The VAS has been identified as the most frequently used pain scale in a systematic review of endometriosis pain scales.[33]
C. Quality of life was assessed with the Endometriosis Health Profile 30 (EHP-30) questionnaire. The EHP-30 is a validated tool that consists of thirty items to assess the 5 key domains of pain, control and powerlessness, social support, emotional well-being, and self-image.[34] The score is standardized on a scale of zero to one hundred, where zero indicates the best health status and one hundred reflects the worst health status.

In addition to patient reported outcome measures, blood was drawn to assess inflammatory markers at the start and end of each of their six study menstrual cycle. The first blood draw was within twenty-four hours of their period starting and prior to starting injections. The second blood draw was within twenty-four hours after the last injection at the end of menses. The inflammatory markers evaluated were thought to be of interest in endometriosis and included: interleukins (IL-1B (beta), IL-1RA (receptor antagonist), IL-2 IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13), Monocyte Chemoattractant Protein (MCP), Tumor Necrosis Factor-alpha (TNF- α), Brain Derived Neurotrophic Factor (BDNF),

Cancer Antigen-125 (CA-125), C-Reactive Protein (CRP), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), and Interferon Gamma (IFN-γ).

Measures of Interest

The primary outcome of interest was improvement in dysmenorrhea. This was primarily assessed using the dysmenorrhea specific portion of the modified B&B questionnaire completed monthly at the end of each period. The score was averaged over three months of anakinra compared to the same analysis during three months of placebo. We hypothesized that lower dysmenorrhea scores via the modified B&B would be reported during cycles on anakinra compared to those when on placebo. Dysmenorrhea was also assessed with daily VAS and comparing scores for treatment versus placebo cycles. Secondary outcomes included changes in quality of life assessed using the EHP-30 questionnaire, changes in menstrual cycle length as an indirect marker of ovulatory dysfunction, and inflammatory biomarkers.

Statistical Analysis

For the primary analysis, participants who provided survey responses in at least one cycle under each treatment condition were included. Baseline characteristics between treatment groups were compared using *t*-test or Fisher's exact test, as appropriate. Mean pain scores and menstrual cycle characteristics were compared between treatment conditions using paired *t*-test. All analyses were conducted using SAS 9.4.

For the quantification of circulating proteins, BDNF (R&D Systems), CRP (RayBiotech), and CA-125 (R&D Systems) were quantified in duplicate in plasma by ELISA according to manufacturer's protocols. All remaining analytes previously listed were quantified using the human 15-plex cytokine assay (Eve Technologies, Calgary AB). For all subjects, the first blood draw on each treatment, whether anakinra or placebo, was excluded in analysis as the treatment had yet to be administered. Data were assessed for normality using the Kolmogorov–Smirnov test. Parametric (Mann–Whitney, *t*-test) or non-parametric (Wilcoxon test; Wilcoxon Matched-Pairs Signed Rank Test) tests were performed in GraphPad Prism (v10.2.3; GraphPad/Dotmatics, Boston, MA) to determine statistically significant differences between study groups. Statistical tests employed are denoted in the text and figure legends Data are presented as mean ± standard error, unless otherwise indicated. A p-value of <0.05 was considered significant for all analyses.

Results

Initial recruitment for this study was compromised by the onset of the global coronavirus-19 (COVID) pandemic as some patients no longer wanted to take an immune suppressor, expressed vaccine hesitancy, or lacked a clear understanding of COVID. In addition, some either did not want or were not permitted to visit healthcare facilities for non-critical blood draws. All of these reasons negatively impacted recruitment. Of the 35 women that were consented for this study, 4 dropped out due to injection reactions, 4 changed their mind and elected for established management including surgery, 2 moved out of the area, 5 dropped out for undisclosed reasons, and 3 were lost to follow up. The remaining 17 subjects completed all treatments, of which 15 had complete questionnaires for both treatments. Seven of these participants were randomized to Treatment Group A and received anakinra for the first three periods and placebo for the subsequent 3 periods, while eight patients in Treatment Group B received placebo and then anakinra. There were no significant differences in the demographics between the two groups (Table 1). Three participants completed all components of the study.

Questionnaire Analysis

In this pilot study, mean B&B dysmenorrhea scores were decreased but not statistically different in anakinra treatment cycles compared to placebo (1.4 vs 1.6, $p = 0.40$) (Table 2). Mean dysmenorrhea VAS trended toward improvement with a score of 37.5 during active treatment compared to 42.6 with placebo ($p = 0.26$). All domains of the EHP-30 also showed a trend towards improvement, with statistically significant improvements noted in the powerlessness (54.5 vs 63.3, $p = 0.04$) and self-image (58.1 vs 66.7, $p = 0.03$) sub-scales while on study drug compared to placebo (Table 2).

Regarding impact on menstrual cycles and ovulation, there was a non-statistical trend toward shorter periods on anakinra (5.0 days vs 5.3 days, $p = 0.28$), but no significant increase in menstrual cycle length (29.3 days vs 27.7 days, $p = 0.56$). Importantly for those attempting conception, women still had periods and hence the suggestion that ovulation

Table 1 Participant Demographics: Treatment Group A Received Anakinra During the First Three Periods and Placebo for the Remaining Three. Treatment B Received Placebo for the First Three Periods and Anakinra for the Subsequent Three

Characteristic	Overall	Treatment Group		p-Value[a]
		A n = 7	B n=8	
Age at enrollment				0.30
Mean (SD)	34.2 (5.5)	32.6 (6.6)	35.6 (4.4)	
Median (IQR)	32 (30, 41)	30 (29, 41)	36 (32, 40)	
Ethnicity, n (%)				0.81
Hispanic	4 (26.7)	2 (28.6)	2 (25.0)	
Non-Hispanic	6 (40.0)	2 (28.6)	4 (50.0)	
Unknown	5 (33.3)	3 (42.9)	2 (25.0)	
Race, n (%)				0.13
White	8 (53.3)	2 (28.6)	6 (75.0)	
Asian	1 (6.7)	0 (0.0)	1 (12.5)	
Mixed	3 (20.0)	2 (28.6)	1 (12.5)	
Unknown	3 (20.0)	3 (42.9)	0 (0.0)	
Baseline dysmenorrhea (B&B)				0.47
Mean (SD)	2.5 (0.5)	2.4 (0.5)	2.6 (0.5)	
Median (IQR)	3 (2, 3)	2 (2, 3)	3 (2, 3)	

Notes: N = 15 patients with at least 1 follow-up survey in each treatment period. [a]p-value based on *t*-tests or Fisher's exact test, as appropriate.
Abbreviations: SD, Standard deviation; IQR, Interquartile range.

Table 2 Questionnaire Outcomes and Menstrual Cycle Changes on Treatment versus Placebo

Scale	Anakinra Mean (SD)	Placebo Mean (SD)	p-Value[a]
Modified Dysmenorrhea (B&B)	1.4 (0.9)	1.6 (0.7)	0.40
VAS (Dysmenorrhea)	37.5 (22.3)	42.6 (19.4)	0.26
EHP-30 (Quality of Life)			
Pain	46.3 (14.2)	49.4 (11.5)	0.21
Control and powerlessness	**54.5 (23.5)**	**63.3 (19.8)**	**0.04**
Emotional well-being	39.1 (18.8)	44.7 (17.5)	0.20
Social support	45.1 (30.0)	50.8 (22.5)	0.11
Self-image	**58.1 (28.6)**	**66.7 (23.0)**	**0.03**
Number of bleeding days[b]	5.0 (1.7)	5.3 (1.3)	0.28
Menstrual cycle length[c]	29.3 (7.4)	27.7 (3.3)	0.56

Notes: N = 15 patients with at least 1 follow-up survey in each treatment period. Bolded numbers indicate statistical significance p<0.05. For all scales, higher score = worse pain. [a] p-value based on paired *t*-tests for mean scores averaged across treatment cycles. [b] n = 12 patients with at least 1 period length in each treatment period. [c] n = 7 patients with at least 1 cycle length in each treatment period.
Abbreviation: SD, Standard deviation.

was still taking place when receiving anakinra. These numbers reflect the twelve and seven patients who had at least one period length and at least one cycle length recorded in each treatment condition, respectively.

Biomarker Analysis

Three subjects per treatment group completed all blood draws for all 6 period cycles allowing for paired analyses. Among these subjects, in Treatment Group A, BDNF levels were significantly lower during active treatment in Cycles 1–3 than placebo during Cycles 4–6 ($p = 0.0001$) (Figure 1). BDNF levels for Treatment Group B were also significantly lower during active treatment in Cycles 4–6 than during placebo in Cycles 1–3 ($p = 0.0350$). These Mann Whitney analyses exclude the first lab draw of the treatment and placebo groupings (excluding pre-cycle 1 and pre-cycle 4 BDNF levels) as there would not be any effect from the intervention at that time.

Analysis of *all* participants' BDNF data, even those without blood draws for all 6 cycles, still revealed a trend towards lower BDNF levels during active treatment compared to placebo for Group A ($p = 0.0682$) (Figure 2). This trend was not seen for Group B that received the study drug for 3 months prior to the 3 months with placebo ($p = 0.6895$). This

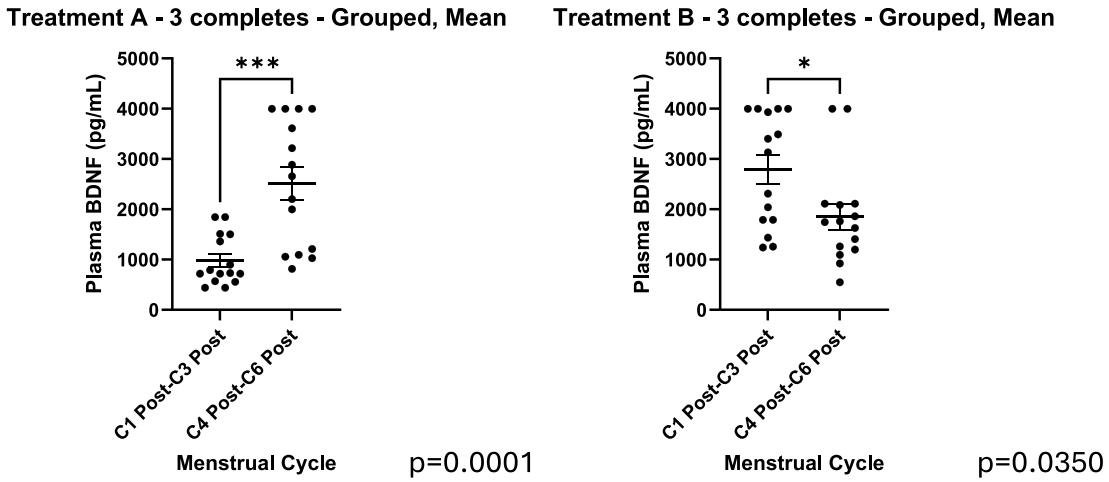

Figure 1 Group A and B BDNF Levels: Paired Data. * Denotes $p \leq 0.05$ and *** denotes $p \leq 0.001$.

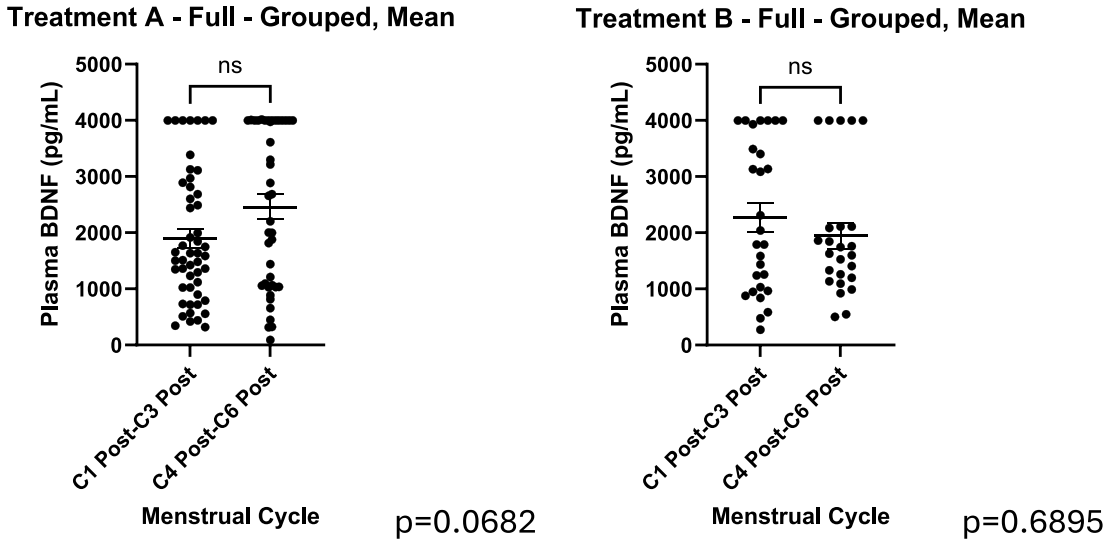

Figure 2 Group A and B BDNF Levels: All Participants' Data.

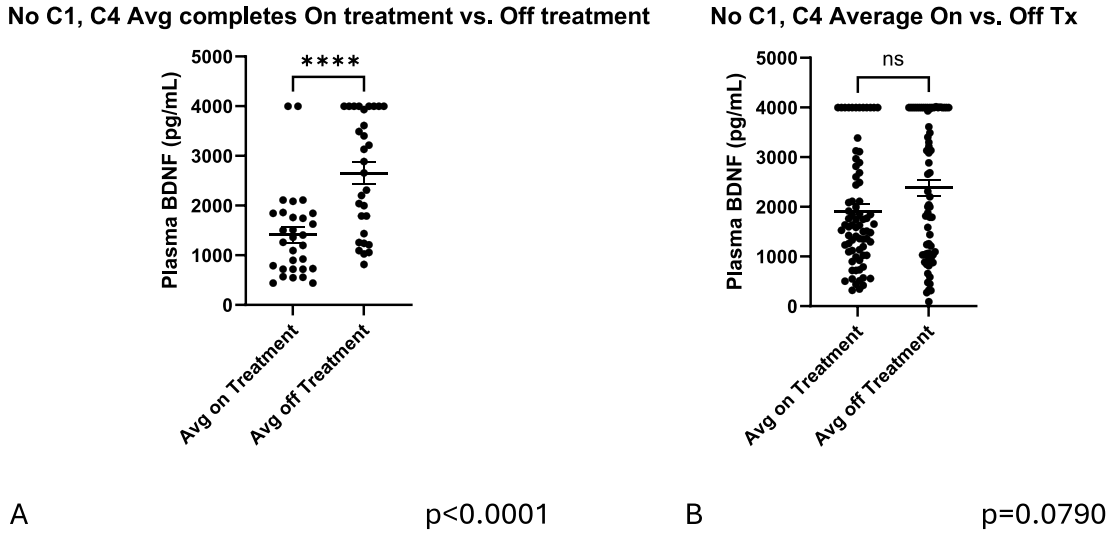

Figure 3 Wilcoxon Matched-Pairs Signed Rank Test Analysis of Average BDNF Levels. (**A**) Participants with Complete Blood Draws for 6 Menstrual Cycles, **** Denotes p ≤ 0.0001. (**B**) All Participants, ns denotes p >0.05 (no significance).

was an unpaired analysis as there were missing paired datasets. As above, this analysis excluded the pre-cycle 1 and pre-cycle 4 data.

When the 6 subjects who completed blood draws for all 6 period cycles are compared using the alternate Wilcoxon Matched-Pairs Signed Rank Test, the average BDNF level on active treatment is significantly lower than on placebo ($p < 0.0001$) (Figure 3A). When all participants are included, not only the 6 who completed all blood draws for all cycles, there is still a trend towards significance ($p = 0.0790$, Figure 3B). Like above, this analysis excluded the pre-cycle 1 and pre-cycle 4 data.

We evaluated multiple other biomarkers with possible roles in endometriosis, but few showed significance using Mann–Whitney analyses (Table 3). These analyses included all available data and not only those with complete data sets. When evaluating IL-1RA levels, there was a significant difference between those on and off treatment in Group B (p =

Table 3 Additional Biomarker Analysis

Analyte	Group A			Group B			Combined		
	On Treatment	Off Treatment	p Value	On Treatment	Off Treatment	p Value	On Treatment	Off Treatment	p Value
CA-125 (U/mL)	6.36±1.08	9.78±1.78	0.2883	8.40±2.20	8.82±2.34	0.8416	7.20±1.06	9.23±1.37	0.3593
CRP (pg/mL)	3434±1018	5648±1339	0.2884	2866±1019	3381±842.5	0.3391	3242±751.7	4750±881.5	0.1842
GM-CSF (U/mL)	74.7±25.76	94.12±27.38	0.5013	41.89±14.63	40.39±12.76	0.8125	62.98±17.39	73.46±17.81	0.6907
IFN-g (pg/mL)	1.69±0.54	2.462±0.74	0.7238	2.05±1.05	2.54±1.11	0.9626	1.81±0.50	2.49±0.62	0.9004
IL-1B (pg/mL)	11.28±3.86	10.86±4.16	0.7401	17.38±7.29	18.56±8.19	0.5773	13.39±3.55	13.82±4.04	0.5669
IL-1RA (pg/mL)	3612±1027	43.05±27.0	0.3999	5093±1983	8.70±2.44	**0.0048**	4123±953.6	29.84±16.7	**0.0222**
IL-2 (pg/mL)	1.88±0.79	2.24±0.97	0.9456	3.61±1.82	4.34±2.07	0.9266	2.47±0.81	3.07±1.01	0.88
IL-4 (pg/mL)	0.76±0.30	0.88±0.39	0.4053	1.22±0.56	1.43±0.61	0.9737	0.91±0.27	1.1±0.33	0.478
IL-5 (pg/mL)	3.87±0.80	3.36±0.82	0.6479	9.03±3.79	10.00±4.20	0.4892	5.62±1.41	5.99±1.77	0.4527
IL-6 (pg/mL)	1.28±0.40	1.70±0.40	**0.0283**	1.35±0.44	1.48±0.48	0.5973	1.31±0.30	1.61±0.30	**0.0475**
IL-8 (pg/mL)	2.56±0.55	2.53±0.43	0.5329	3.31±0.93	4.43±0.94	0.3044	2.81±0.48	3.26±0.46	0.2117
IL-10 (pg/mL)	3.38±0.62	3.99±0.88	0.8438	8.21±4.02	10.55±4.95	0.7605	5.01±1.43	6.64±2.09	0.4982
IL-12p40 (pg/mL)	123.7±54.92	102.9±23.93	0.745	137.1±52.94	146±62.83	0.4553	128.4±40.07	119.4±28.08	0.7259
IL-12p70 (pg/mL)	4.19±1.44	5.03±1.83	0.7622	15.78±7.53	17.3±8.55	0.935	7.99±2.704	9.749±3.52	0.7427
IL-13 (pg/mL)	40.02±12.2	46.64±14.89	0.9468	85.02±26.41	70.95±24.61	0.3772	55.54±12.31	56.28±13.22	0.6446
MCP-1 (pg/mL)	166.4±13.67	153.6±10.53	0.476	140.4±9.1	140.7±9.90	0.9786	157.9±9.743	148.7±7.50	0.4624
TNF-a (pg/mL)	18.03±2.54	18.04±3.10	0.6968	44.58±15.2	49.77±18.72	0.8013	27.18±5.67	30.61±7.84	0.8796

Notes: Bolded numbers indicate statistical significance by Mann–Whitney or *t*-test, p<0.05.

0.0048) as well as between those on and off treatment for Groups A and B combined (p = 0.0222). For IL-6, there was a significant difference between those on and off treatment in Group A (p = 0.0283) and for Group A and B combined (p = 0.0475).

Mean values within patient under both treatment conditions were utilized to ensure inclusion of as many patients as possible. Given the pilot nature of this study, no imputations were done for individual missing values, which may have reduced our power to detect differences. Dropout occurred early in both randomization cycles; thus, missing data is unlikely to be related to treatment assignment.

Discussion

In this randomized, placebo-controlled, cross-over pilot study of women with endometriosis, the IL-1 receptor inhibitor anakinra led to a non-statistically significant decrease in dysmenorrhea pain scores as assessed by the B&B questionnaire and VAS scores. Analysis of EHP-30 domains suggested improvements in quality of life. Also of clinical importance is that women still had monthly periods when on anakinra, which is important for those attempting conception. Inflammatory markers of endometriosis trended down with anakinra, with BDNF being significantly lower.

Questionnaire Discussion

Non-significant decreases in pain scores were noted for women on anakinra as measured by B&B dysmenorrhea and VAS scores. Although the decrease was insignificant in this small pilot study, pain is one of the primary clinical targets for treatment for endometriosis and potential effectiveness of this medication should be assessed in a larger trial. As well as assessing dysmenorrhea, future studies will need to assess the impact of anakinra on dyspareunia and non-menstrual pain, which are also common with endometriosis. All 5 domains of the EHP 30 showed a tendency for improvement with anakinra, with significant improvements being detected in the domains of level of control patients had regarding their disease and general self-image. Since improving pain without improving overall quality of life is likely of limited value, it is reassuring that quality of life improved as well as pain.

Menstrual Cycle Discussion

Demonstrating that patients on anakinra do not have altered menstrual cycles is a crucial first step in confirming preservation of fertility potential. The preservation of menstrual cycle length is an indication that ovulation is not impaired with anakinra. It is potentially one of the major advantages of this therapy over all current endometriosis therapeutics. All FDA-approved medications for endometriosis are hormonal agents that typically produce a state of hypoestrogenism which precludes pregnancy and limits length of use despite endometriosis being a disease spanning several decades. Anakinra's safety profile with respect to fertility and pregnancy, as indicated by both animal and limited human studies, suggests minimal risk. Animal studies showed no evidence of fetal harm or impact on reproductive capacity.[29] With regard to teratogenicity, it is classified as pregnancy Category B. A systematic review found IL-1 antagonism was not associated with increased adverse perinatal outcomes and multiple case reports of women taking anakinra during pregnancy and while breastfeeding noted no complications.[35,36]

Biomarker Discussion

Although we expected to find the most robust decreases in IL-1, we identified that BDNF levels were significantly decreased with anakinra. Circulating levels of BDNF have previously been linked with endometriosis and is one of the key biomarkers shown to correlate with inflammation in the disease process and pain of endometriosis.[9,37]. For subjects with complete biomarker datasets, paired comparison of the BDNF levels showed a statistically significant decrease during treatment with anakinra and further suggests that anakinra drives a decrease in inflammation. However, when data from all subjects with any blood draws were analyzed, BDNF levels trended down but did not reach statistical significance with anakinra treatment. We propose that this is due to the inability to conduct paired tests as BDNF levels are known to vary substantially between individuals. Furthermore, decreased sample size could hide potential associations. Building on this, some of the lack of significance could be accounted for by a continued impact of anakinra despite its relatively short half-life of 4–6 hours given blood draws one month after administration showed continued suppression

of BDNF levels. As such, the impact of treatment appeared to continue beyond the timeframe during which the drug was administered.

IL-1RA and IL-6 were shown to be significantly reduced when Groups A and B were combined and in Group B and A, respectively. Anakinra is a recombinant form of IL-1RA and acts by inhibiting IL-1 receptor, but it is unknown if IL-1 follows traditional negative feedback loop physiology, limiting the analysis of the trend in IL-1RA and Il-1B. Of note, there was not a significant decrease in IL-1 B. The inhibition of IL-1 through anakinra treatment physiological decreases inflammation, as seen in rheumatoid arthritis. IL-8, another potential interleukin associated in endometriosis pathophysiology, was not shown to be statistically decreased in this study. Given that each of the biomarkers assessed trended lower with anakinra, with varying significance depending on the grouping, we predict that a larger study would likely find these changes statistically significant and confirm the anti-inflammatory nature of this treatment.

Strengths of this pilot study include the randomized, double-blind, cross-over design of women with endometriosis in which each patient serves as their own control. Limitations of this pilot study are secondary to the small samples size and participant drop out in part due to the pandemic. This limited our analysis given we did not have complete paired data for all questionnaires or biomarkers throughout the 6 menstrual cycles. Although our group has conducted several endometriosis-related clinical trials in the past and we understand the challenges studying this group of patients, especially when a placebo is involved, we were unprepared for the global fear brought about by the pandemic which occurred soon after starting this study. Presumably due to the natural concerns about taking an immune suppressant at a time that they were vulnerable to contracting COVID-19, together with inability or reluctance to attend healthcare facilities for their blood draws, many patients who had consented to participate either declined to continue or were lost to follow up. Our limitations highlight the need for a larger, multi-center study, which may find that some of our non-statistically significant findings become significant. Furthermore, although this was a double-blind study, there was the possibility of observer bias such as if participant thought they knew they were receiving the study drug and perceived they experienced more relief as a result.

Given the positive clinical trends and decrease in inflammatory biomarkers, further study for the treatment of endometriosis with anakinra could be beneficial for the many patients suffering from this disease. Fertility rates of anakinra-treated patients with endometriosis could be clinically investigated due to its significant advantage over other therapies. Additionally, a future step would be to examine mechanisms specific to endometriosis that allow anakinra to potentially decrease inflammation and pain beyond the treatment window, thus possibly reducing dosing to less than daily during menses. Future studies could also examine if the decrease in inflammation can limit or reverse the disease process and adhesions, as seen with IL-8 antagonism. Studies should also evaluate patient willingness to self-administer a subcutaneous medication, particularly given 4 patients dropped out due to injection reactions. Anakinra is unique from the current medications approved for endometriosis both in mechanism of action as well as its delivery mechanism and requires additional evaluation. As endometriosis has multiple behaviors that are similar to malignant lesions, including decreased apoptosis, neo-angiogenesis, and cellular invasion, and is associated with increased risk of ovarian cancer, we must also evaluate how any new therapies may impact risk of malignancy.[38–40]

Conclusion

Endometriosis is a significant global health concern affecting up to 10% of women worldwide causing significant pelvic pain, infertility, and other impacts on daily life. In this pilot study, anakinra demonstrated clinical and physiological potential warranting a larger and more comprehensive evaluation as a long term, non-hormonal therapeutic targeting endometriosis related pain and inflammation without disrupting fertility.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author, Dr. Sanjay Agarwal, upon reasonable request.

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Disclosure

Dr Foster and Dr Wessels are Co-Founders of Afynia Laboratories, a medical diagnostic company, and hold several patents unrelated to the treatment of endometriosis. The other authors report no conflicts of interest in this work.

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References

1. Agarwal SK, Chapron C, Giudice LC, et al. Clinical diagnosis of endometriosis: A call to action. *Am J Obstet Gynecol*. 2019;220(4):354e1–354e12. doi:10.1016/j.ajog.2018.12.039
2. Della Corte L, Di Filippo C, Gabrielli O, et al. The burden of endometriosis on women's lifespan: A narrative overview on quality of life and psychosocial wellbeing. *Int J Environ Res Pub Health*. 2020;17(13):4683. doi:10.3390/ijerph17134683
3. Skegro B, Bjedov S, Mikus M, et al. Endometriosis, pain and mental health. *Psychiatry Danub*. 2021;33(Suppl 4):632–636.
4. Mikus M, Vitale SG, Coric M, et al. State of the art, new treatment strategies, and emerging drugs for non-hormonal treatment of endometriosis: A systematic review of randomized control trials. *Gynecol Endocrinol*. 2022;38(11):911–917. doi:10.1080/09513590.2022.2133105
5. Zajec V, Mikus M, Vitale SG, et al. Current status and challenges of drug development for hormonal treatment of endometriosis: A systematic review of randomized control trials. *Gynecol Endocrinol*. 2022;38(9):713–720. doi:10.1080/09513590.2022.2109145
6. Newmark AL, Luciano DE, Ulrich A, Luciano AA. Medical management of endometriosis. *Minerva Obstet Gynecol*. 2021;73(5):572–587. doi:10.23736/S2724-606X.21.04776-X
7. Della Corte L, Barra F, Mercorio A, et al. Tolerability considerations for gonadotropin-releasing hormone analogues for endometriosis. *Expert Opin Drug Metab Toxicol*. 2020;16(9):759–768. doi:10.1080/17425255.2020.1789591
8. Sacco K, Portelli M, Pollacco J, Schembri-Wismayer P, Calleja-Agius J. The role of prostaglandin E2 in endometriosis. *Gynecol Endocrinol*. 2012;28(2):134–138. doi:10.3109/09513590.2011.588753
9. Yu J, Francisco AMC, Patel BG, et al. IL-1beta stimulates brain-derived neurotrophic factor production in eutopic endometriosis stromal cell cultures: A model for cytokine regulation of neuroangiogenesis. *Am J Pathol*. 2018;188(10):2281–2292. doi:10.1016/j.ajpath.2018.06.011
10. Zeitoun KM, Bulun SE. Aromatase: A key molecule in the pathophysiology of endometriosis and a therapeutic target. *Fertil Steril*. 1999;72(6):961–969. doi:10.1016/s0015-0282(99)00393-3
11. Bulun SE, Yildiz S, Adli M, et al. Endometriosis and adenomyosis: Shared pathophysiology. *Fertil Steril*. 2023;119(5):746–750. doi:10.1016/j.fertnstert.2023.03.006
12. Bulun SE, Zeitoun K, Takayama K, et al. Estrogen production in endometriosis and use of aromatase inhibitors to treat endometriosis. *Endocr Relat Cancer*. 1999;6(2):293–301. doi:10.1677/erc.0.0060293
13. Mikus M, Goldstajn MS, Brlecic I, et al. CTLA4-linked autoimmunity in the pathogenesis of endometriosis and related infertility: A systematic review. *Int J Mol Sci*. 2022;23(18). doi:10.3390/ijms231810902
14. Kyama CM, Mihalyi A, Simsa P, et al. Non-steroidal targets in the diagnosis and treatment of endometriosis. *Curr Med Chem*. 2008;15(10):1006–1017. doi:10.2174/092986708784049595
15. Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: Origins, consequences and interventions. *Acta Obstet Gynecol Scand*. 2017;96(6):623–632. doi:10.1111/aogs.13156
16. Sikora J, Ferrero S, Mielczarek-Palacz A, Kondera-Anasz Z. The delicate balance between the good and the bad IL-1 proinflammatory effects in endometriosis. *Curr Med Chem*. 2018;25(18):2105–2121. doi:10.2174/0929867325666180111093547
17. Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z. Imbalance in cytokines from interleukin-1 family - role in pathogenesis of endometriosis. *Am J Reprod Immunol*. 2012;68(2):138–145. doi:10.1111/j.1600-0897.2012.01147.x
18. Ruscitti P, Cipriani P, Liakouli V, et al. The emerging role of IL-1 inhibition in patients affected by rheumatoid arthritis and diabetes. *Rev Recent Clin Trials*. 2018;13(3):210–214. doi:10.2174/1574887113666180314102651
19. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev*. 2018;281(1):8–27. doi:10.1111/imr.12621
20. Zondervan KT, Becker CM, Missmer SA. Endometriosis. *N Engl J Med*. 2020;382(13):1244–1256. doi:10.1056/NEJMra1810764
21. Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z. Association of the precursor of interleukin-1beta and peritoneal inflammation-role in pathogenesis of endometriosis. *J Clin Lab Anal*. 2016;30(6):831–837. doi:10.1002/jcla.21944
22. Werdel R, Mabie A, Evans TL, et al. Serum levels of interleukins in endometriosis patients: A systematic review and meta-analysis. *J Minim Invasive Gynecol*. 2024;31(5):387–396e11. doi:10.1016/j.jmig.2024.02.011
23. Cohen SB, Moreland LW, Cush JJ, et al. A multicentre, double blind, randomised, placebo controlled trial of anakinra (Kineret), a recombinant interleukin 1 receptor antagonist, in patients with rheumatoid arthritis treated with background methotrexate. *Ann Rheum Dis*. 2004;63(9):1062–1068. doi:10.1136/ard.2003.016014
24. Cavalli G, Dinarello CA. Anakinra therapy for non-cancer inflammatory diseases. *Front Pharmacol*. 2018;9:1157. doi:10.3389/fphar.2018.01157
25. Mohamed Hussein AAR, Sayad R, Abdelshafi A, et al. A meta analysis on the utility of Anakinra in severe COVID-19 disease. *Cytokine*. 2023;169:156311. doi:10.1016/j.cyto.2023.156311
26. Taddio A, Della Paolera S, Abbagnato L, et al. Early anakinra treatment improves cardiac outcome of multisystem inflammatory syndrome in children, regardless of disease severity. *Rheumatol*. 2024;63(2):366–375. doi:10.1093/rheumatology/kead381
27. Vallejo S, Palacios E, Romacho T, Villalobos L, Peiro C, Sanchez-Ferrer CF. The interleukin-1 receptor antagonist anakinra improves endothelial dysfunction in streptozotocin-induced diabetic rats. *Cardiovasc Diabetol*. 2014;13:158. doi:10.1186/s12933-014-0158-z
28. Hallegua DS, Weisman MH. Potential therapeutic uses of interleukin 1 receptor antagonists in human diseases. *Ann Rheum Dis*. 2002;61(11):960–967. doi:10.1136/ard.61.11.960
29. Food and Drug Administration. Highlights of Prescribing Information: Kineret; 2012. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2012/103950s5136lbl.pdf. Accessed 15, January 2023.

30. Whitehead AL, Julious SA, Cooper CL, Campbell MJ. Estimating the sample size for a pilot randomised trial to minimise the overall trial sample size for the external pilot and main trial for a continuous outcome variable. *Stat Methods Med Res*. 2016;25(3):1057–1073. doi:10.1177/0962280215588241
31. Kunselman AR. A brief overview of pilot studies and their sample size justification. *Fertil Steril*. 2024;121(6):899–901. doi:10.1016/j.fertnstert.2024.01.040
32. Biberoglu KO, Behrman SJ. Dosage aspects of danazol therapy in endometriosis: Short-term and long-term effectiveness. *Am J Obstet Gynecol*. 1981;139(6):645–654. doi:10.1016/0002-9378(81)90478-6
33. Bourdel N, Alves J, Pickering G, Ramilo I, Roman H, Canis M. Systematic review of endometriosis pain assessment: How to choose a scale? *Hum Reprod Update*. 2015;21(1):136–152. doi:10.1093/humupd/dmu046
34. Jones G, Kennedy S, Barnard A, Wong J, Jenkinson C. Development of an endometriosis quality-of-life instrument: The endometriosis health profile-30. *Obstet Gynecol*. 2001;98(2):258–264. doi:10.1016/s0029-7844(01)01433-8
35. National Institutes of Health. Anakinra; 2023. Available from: https://www.ncbi.nlm.nih.gov/books/NBK501871/. Accessed 24, July 2024.
36. Brien ME, Gaudreault V, Hughes K, Hayes DJL, Heazell AEP, Girard S. A systematic review of the safety of blocking the IL-1 system in human pregnancy. *J Clin Med*. 2021;11(1):225. doi:10.3390/jcm11010225
37. Wessels JM, Kay VR, Leyland NA, Agarwal SK, Foster WG. Assessing brain-derived neurotrophic factor as a novel clinical marker of endometriosis. *Fertil Steril*. 2016;105(1):119–28e1–5. doi:10.1016/j.fertnstert.2015.09.003
38. Nasu K, Nishida M, Kawano Y, et al. Aberrant expression of apoptosis-related molecules in endometriosis: A possible mechanism underlying the pathogenesis of endometriosis. *Reprod Sci*. 2011;18(3):206–218. doi:10.1177/1933719110392059
39. Barnard ME, Farland LV, Yan B, et al. Endometriosis typology and ovarian cancer risk. *JAMA*. 2024;332(6):482–489. doi:10.1001/jama.2024.9210
40. Chung MS, Han SJ. Endometriosis-associated angiogenesis and anti-angiogenic therapy for endometriosis. *Front Glob Womens Health*. 2022;3:856316. doi:10.3389/fgwh.2022.856316