Original Research

Evaluation of the Effects of Neptune Krill Oil on Chronic Inflammation and Arthritic Symptoms

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Journal of the American College of Nutrition, Vol. 26, No. 1, 39–48 (2007)
Published by the American College of Nutrition

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Key words: C-reactive protein, inflammation, omega-3, phospholipids, Neptune Krill Oil, NKO™, antioxidants

Objectives: a) To evaluate the effect of Neptune Krill Oil (NKO™) on C-reactive protein (CRP) on patients with chronic inflammation and b) to evaluate the effectiveness of NKO™ on arthritic symptoms.

Methods: Randomized, double blind, placebo controlled study. Ninety patients were recruited with confirmed diagnosis of cardiovascular disease and/or rheumatoid arthritis and/or osteoarthritis and with increased levels of CRP (>1.0 mg/dl) upon three consecutive weekly blood analysis. Group A received NKO™ (300 mg daily) and Group B received a placebo. CRP and Western Ontario and McMaster Universities (WOMAC) osteoarthritis score were measured at baseline and days 7, 14 and 30.

Results: After 7 days of treatment NKO™ reduced CRP by 19.3% compared to an increase by 15.7% observed in the placebo group ($p = 0.049$). After 14 and 30 days of treatment NKO™ further decreased CRP by 29.7% and 30.9% respectively ($p < 0.001$). The CRP levels of the placebo group increased to 32.1% after 14 days and then decreased to 25.1% at day 30. The between group difference was statistically significant; $p = 0.004$ at day 14 and $p = 0.008$ at day 30. NKO™ showed a significant reduction in all three WOMAC scores. After 7 days of treatment NKO™, reduced pain scores by 28.9% ($p = 0.050$), reduced stiffness by 20.3% ($p = 0.001$) and reduced functional impairment by 22.8% ($p = 0.008$).

Conclusion: The results of the present study clearly indicate that NKO™ at a daily dose of 300 mg significantly inhibits inflammation and reduces arthritic symptoms within a short treatment period of 7 and 14 days.

INTRODUCTION

Inflammation is closely linked to the pathogenesis of atherosclerosis and joint disease and may be provoked by noninfectious (e.g., injury, smoking, diabetes, obesity) as well as infectious sources. C-reactive protein (CRP), which is one of the most useful biomarkers of inflammation, appears to be a central player in the harmful effects of systemic inflammation and an easy and inexpensive screening test to assess inflammation-associated risk [1]. Unlike other markers of inflammation, CRP levels are stable over long periods, have no diurnal variation and can be measured inexpensively.

Current studies suggest that CRP is a strong predictor of future cardiovascular events [2–5]. At all levels of estimated 10-year risk for events according to the Framingham risk score and at all levels of LDL cholesterol, CRP remained a strong predictor of future cardiovascular risk [6]. CRP has been shown in several prospective, nested case-control studies to be associated with an increased risk of myocardial infarction [7–12], stroke [7,9,13,14], sudden death from cardiac causes [15], and peripheral arterial disease [16].

In arthritic joints CRP production reflects the release of proinflammatory cytokines, such as interleukins-1 and -6 (IL-1 and IL-6) and tumor necrosis factor-alpha (TNF-α), which are essential in the mechanism of cartilage degeneration [17–22]. CRP is significantly increased in patients with rheumatoid arthritis and slightly but significantly higher in patients with osteoarthritis than in matched controls [1,23–29]. CRP was also found to increase in patients with knee osteoarthritis showing disease progression as well as in patients with rapidly destructive hip osteoarthritis [24–29]. Contrary to erythrocyte sedimentation rate (ESR), evidence has proven a strong association between CRP

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Journal of the American College of Nutrition, Vol. 26, No. 1, 39–48 (2007)
Published by the American College of Nutrition

and level of clinical severity in patients with osteoarthritis of the knee or hip [24,26].

The results of human studies on the anti-inflammatory properties of omega-6 and omega-3 fatty acids are controversial, varying from no effect to a beneficial effect [30–34]. A proposed competition between omega-3 and omega-6 fatty acids may be the reason for the observed discrepancies of the effects of n-3 fatty acids on cytokines [35]. Both omega-3 and omega-6 fatty acids are substrates for the production of human eicosanoids and share the same enzymes for the synthesis of prostaglandins and leukotrienes. Omega-3 fatty acids produce eicosanoids with fewer inflammatory properties than those derived from omega-6 fatty acids [36–38]. Hence, a dominant ratio of dietary intake of omega-3 versus omega-6 fatty acids is critical to inflammatory processes.

Neptune Krill Oil is extracted from Antarctic Krill (*Ephausia Superba*), a zooplankton at the bottom of the food chain. Even though krill is the main food source for whales it remains the most abundant biomass on earth because of its high regeneration properties. The krill used for Neptune Krill Oil is harvested in the Antarctic Ocean where the worldwide harvest is less than 0.1% the allowed fishing quota. Being at the bottom of the food chain, having a very short lifespan of 1–2 years and living in the clean waters of the Antarctic Ocean, makes the krill and thus Neptune Krill Oil naturally pure of heavy metals, dioxins and pesticides.

The oil is extracted by a patented cold vacuum extraction process that protects the biomass from exposure to heat, light or oxygen. This protects the oil through-out its production and maintains the original nutrients of krill intact. The result is a concentrate of novel marine phospholipid carriers of eicosapentanoic (EPA) and docosahexanoic (DHA) fatty acids and potent antioxidants. The main antioxidants, astaxanthin and a novel flavonoid, similar to the 6,8-Di-C-glucosylluteolin, esterify the EPA and DHA respectively. This provides a significant stability and antioxidant potency to the oil.

Anecdotal data suggests that Neptune Krill Oil may be effective for the management of arthritic symptoms. Evidence has shown that phospholipids, omega-3 fatty acids and astaxanthin have direct or indirect anti-inflammatory properties [9–13,30–53]. Phospholipids protect the cell membrane for toxic injury [39]. Multiple studies have proven EPA and DHA to trigger secretion of anti-inflammatory prostaglandins of the 3 series (PE_3, PI_3 and thromboxane A_3) and interleukin-6 resulting in a decrease CRP and tumor necrosis factor (TNF) [30–38,40–53]. Astaxanthin inhibits the production of proinflammatory prostaglandins (PGE2) and TNF [9–13,41–43]. A dietary supplement that contains a natural combination of phospholipids, EPA, DHA and astaxanthin may offer an alternative regimen for the management of chronic inflammatory conditions.

Considering the continuously increasing evidence of adverse events related to the chronic use of non-steroidal anti-inflammatory drugs (NSAIDs), which represent the gold standard for the treatment of chronic inflammatory conditions, it is imperative to research for more innovative and safer treatments [54–61]. The current study addresses the need for safer alternatives in the management of inflammation and arthritic disease and evaluates the hypotheses that Neptune Krill Oil is safe and effective for the reduction of inflammation as measured by serum CRP and the management of pain in patients with arthritic disease. The objectives of the present study were a) to evaluate the effect of NKO™ on CRP in patients with chronic inflammation and b) to evaluate the effect of NKO™ on the quality of life of patients with arthritic disease.

MATERIALS AND METHODS

Patients

Adult patients between 30 and 75 years with a confirmed diagnosis of cardiovascular disease and/or rheumatoid arthritis and/or osteoarthritis and with increased CRP levels at 1.0 mg/dl or more and a standard deviation not higher than 0.05 in three consecutive weekly tests, who fulfilled the inclusion criteria and signed an informed consent form, were included in the study. Excluded from the study were patients who could not restrain from consuming alcohol for the duration of the study, with a history of gastrointestinal perforation or hemorrhage or symptomatic peptic ulcer. Patients with seafood allergy, diabetes or concurrent medical disease or concomitant treatments (including postmenopausal hormones) that could confound or interfere with the outcome measures, as well as those taking concomitant anticoagulants were not eligible for enrollment. Also excluded were patients with moderate or severe depression or who were unable to respond to the study questionnaire. Women of childbearing age were required to have confirmed use of adequate contraception since their last menses and to agree to continue this practice during the study.

Study Design

In this prospective randomized double blind clinical trial 90 patients who fulfilled the study criteria were recruited from the practices of primary care physicians in Ontario, Canada. Patients were randomly assigned by a computer-generated schedule into one of two groups. The first group (Group A) received NKO™ at a daily morning dose of 300 mg; the second group (Group B) received a neutral placebo. The NKO™ contained 17% EPA, 10% DHA and an omega-3 versus omega-6 ratio of 15 to 1. The placebo used was microcrystalline cellulose. Both the NKO™ and the placebo were administered in non-distinguishable glycerin softgels. Compliance was tested by a count of softgels at each visit after 7, 14 and 30 days. All blood tests were taken at a central lab in the morning, between 7:00 and

10:00 am under fasting conditions for 8 hours. Blood sampling and testing occurred weekly during the 3 week screening period, at baseline after the 1 week wash-out and at each follow-up visit after 7, 14 and 30 days of treatment.

Patients were asked to stop use of all dietary supplements, especially those containing omega-6, foods containing a high content of omega-6 (corn, soy, safflower and sunflower oils and sunflower seeds) and all analgesics (except acetaminophen) and anti-inflammatory medications for two weeks prior to initiation of the trial for washout purposes. Patients were allowed to take acetaminophen (650 mg caplets) as a rescue analgesic medication, for severe pain throughout the trial. The maximum dose of acetaminophen allowed was as recommended by the manufacturer; 1–2 capsules every 8 hours. All patients were instructed to keep a diary of their acetaminophen consumption and report it at their next scheduled visit.

Ninety patients were recruited, 45 per group, of whom 44 patients in the NKO™ group and 43 patients in the placebo group completed 30 days of treatment. Two patients withdrew from the study, one per group, after a minor accident that required additional analgesic treatment. One patient on placebo withdrew for personal reasons. The mean age of patients in the NKO™ group was 54.6 (14.8) years and 55.3 (14.3) years in the placebo group. There were 25 (55.6%) male patients in the NKO™ group and 22 (48.9%) in the placebo group.

In Group A and B respectively, 5 and 7 patients were diagnosed only with atherosclerosis, 18 and 16 patients only with osteoarthritis, 10 and 12 patients only with rheumatoid arthritis and 12 and 10 patients with cardiovascular disease and osteoarthritis. Overall, in the NKO™ and placebo group respectively, 17 and 17 patients were diagnosed with cardiovascular disease, 30 and 26 with osteoarthritis and 10 and 12 with rheumatoid arthritis.

Outcome Measures

During the screening period, in order to avoid the inclusion of patients with acute inflammation, the primary efficacy parameter, C-reactive protein, was measured weekly for three consecutive weeks. Patients who maintained a CRP of at least 1 mg/dl, without fluctuations higher than 0.05 mg, were blindly randomized in their group and after the washout period initiated their respective treatment, either NKO™ or placebo. CRP was further tested after 7, 14 and 30 days of treatment.

At baseline as well as at each of the three follow-up visits, patients with arthritic disease were asked to complete the Western Ontario and McMaster Universities (WOMAC) arthritic pain assessment questionnaire. The Western Ontario and McMaster (WOMAC) University osteoarthritis score is a 24-item questionnaire completed by the patient and focusing on joint pain, stiffness and loss of function related to osteoarthritis of the knee and hip [62–77]. The WOMAC is used extensively in clinical trials for the evaluation of the effect of investigational products on the treatment of osteoarthritis. Even though it was initially developed for the assessment of pain, stiffness and function of daily living in the elderly with osteoarthritis it has recently been revised for younger and/or more active patients with knee injury and/or knee or hip osteoarthritis. It provides a validated assessment of the patient's functional capacity, specifically joint pain, stiffness and functional impairment [62–77]. The WOMAC osteoarthritis score has 3 subscales with 24 items; 5 items assessing pain, 2 items for stiffness, and 17 items measuring physical function. It can be self-administered in less than 5 minutes. The WOMAC can be both scored separately for each subscale and together to give a composite score. The scale employed in this study to quantify patient global assessment of disease activity was the Likert scale; a 5-point scale in which 0 represents the best outcome and 4 the worst. Minimal clinically significant change is considered a decrease of 0.4 mm on each item in the three subscales [71–77]. In order to avoid environmental or other bias, all patients responded to the WOMAC in the physician's office before their examination.

Statistical Design

A sample size of 90 patients (45 patients per group) provided 80% power to detect a CRP reduction of 10% from baseline to 14 days. Within group differences reflecting changes over time for the same patient were assessed for statistical significance with the Paired Student's t-test. Between group differences were assessed with planned comparisons of one way analysis of variance. Statistical significance was set at $p < 0.05$. Values are presented as mean ± standard deviation.

RESULTS

At baseline, there was no significant difference between groups with regards to concomitant medications ($p = 0.987$), CRP levels ($p = 0.087$) and the three WOMAC scores (pain − $p = 0.539$, stiffness − $p = 0.104$, functional impairment − $p = 0.105$). Patients on NKO™ reduced their consumption of rescue medications between baseline and 30 days by 31.6% and significantly less consumption than patients on placebo, who reduced their acetaminophen intake by 5.9% ($p = 0.012$).

After 7 days of treatment NKO™ reduced mean (SD) CRP by 19.3% (1.1) compared to an increase by 15.7% (1.9) observed in the placebo group. The difference between the two groups was statistically significant ($p = 0.049$). NKO™ further decreased CRP after 14 and 30 days of treatment by 29.7% (0.9) and 30.9% (1.0) respectively. The CRP levels of the placebo group increased by 32.1% (1.9) after 14 days and then decreased to 25.1% (1.1) at day 30. The within group decrease of mean (SD) CRP by NKO™ through the three testing periods was statistically significant ($p = 0.001$). Contrary the CRP in the within placebo group increased significantly ($p = 0.028$). The between group difference in all three testing days was

Table 1. C-Reactive Protein (CRP) mg/dl by Group and Visit

			NKO™ 300 mg/day	Placebo	P value (Between Groups)
Visit	Baseline	Mean	2.49	2.87	0.087
		Std Deviation	1.85	1.25	
		Median	2.28	2.83	
	7 Days	Mean	2.01	3.32	0.049
		% Change (Baseline—7 days)	−19.3	15.7	
		Std Deviation	1.08	1.92	
		Median	1.95	3.26	
	14 Days	Mean	1.75	3.79	0.004
		% Change (Baseline—14 days)	−29.7	32.1	
		Std Deviation	0.88	1.88	
		Median	1.86	4.02	
	30 Days	Mean	1.72	3.59	0.008
		% Change (Baseline—30 days)	−30.9	25.1	
		Std Deviation	1.0	1.05	
		Median	1.69	3.44	
P-value (Within Groups)/Interaction			0.001	0.028	

Table 2. WOMAC Pain Scores by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.39	3.07	0.539
		Std Deviation	.91	.60	
		Median	3.19	3.00	
	7 Days	Mean	2.41	2.78	0.052
		Std Deviation	.90	.61	
		Median	2.19	2.71	
	14 Days	Mean	2.52	3.26	0.003
		Std Deviation	.79	.67	
		Median	2.39	3.21	
	30 Days	Mean	2.09	3.05	0.009
		Std Deviation	.85	.59	
		Median	2.02	3.00	
P value (Within Groups)/Interaction			0.002	0.138	

* 0 represents the best outcome and 4 the worst.

statistically significant; $p = 0.049$ at 7 days, $p = 0.004$ at day 14 and $p = 0.008$ at day 30 (Table 1).

Tables 2–7 present the effects of NKO™ on the 3 WOMAC osteoarthritis scores compared to placebo, from baseline to 30 days. NKO™ showed a significant reduction in all three WOMAC scores. NKO™ reduced pain significantly more than placebo in all three follow-up visits; $p = 0.050$ at visit 1 (day 7), $p = 0.049$ at visit 2 (day 14) and $p = 0.011$ at visit 3 (day 30). Similar effects were observed with the stiffness and functional impairment scores. In all three follow-up visits the between group differences in change of stiffness ($p = 0.001$) and functional impairment ($p = 0.005$) were statistically significant (Tables 4–7). No adverse events were reported during the 30 days of treatment with Neptune Krill Oil.

DISCUSSION

Non-steroidal anti-inflammatory agents (NSAIDs) are the most commonly prescribed agents for inflammatory conditions. NSAIDs are drugs with analgesic, antipyretic and anti-inflammatory effects. Most NSAIDs, such as aspirin, ibuprofen and naproxen act as non-selective inhibitors of cyclooxygenase—they inhibit both the cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) isoenzymes, whereas COX-2 inhibitors selectively inhibit the cyclooxygenase-2 isoenzyme. The main advantage of NSAIDs is that, unlike opioids, they do not produce sedation, respiratory depression, or addiction. Certain NSAIDs have become accepted as relatively safe, resulting in the rescheduling of these agents, e.g. ibuprofen, to allow availability over-the-counter. However, recent evidence suggests an

Table 3. Change in WOMAC Pain Scores/100 by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	7 Days	Mean	−28.91	−9.44	0.050
		Std Deviation	18.70	26.98	
		Median	−25.00	−10.00	
		P-Value (Visit)	0.001	0.290	
	14 Days	Mean	−25.66	6.18	0.049
		Std Deviation	15.27	13.54	
		Median	−25.00	.00	
		P-Value (Visit)	0.022	0.208	
	30 Days	Mean	−38.35	−0.6	0.011
		Std Deviation	21.06	15.89	
		Median	−30.00	.00	
		P-Value (Visit)	0.001	0.610	

* 0 represents the best outcome and 4 the worst.

Table 4. WOMAC Stiffness Scores by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.45	2.85	0.104
		Std Deviation	.95	.85	
		Median	3.48	3.02	
	7 Days	Mean	2.75	3.35	0.030
		Std Deviation	.84	.83	
		Median	2.48	3.10	
	14 Days	Mean	2.55	2.83	0.056
		Std Deviation	.79	.99	
		Median	2.50	3.00	
	30 Days	Mean	2.10	2.97	0.043
		Std Deviation	.85	.72	
		Median	2.00	3.01	
	P value (Within Groups)/Interaction		0.002	0.324	

* 0 represents the best outcome and 4 the worst.

association between COX-2 inhibitor exposure and cardiovascular risk. Considering that small increases in ambulatory and clinic systolic blood pressure in patients with hypertension and type II diabetes are associated with substantial increases in the risk of cardiovascular morbidity, the use of these medications has been restricted to the lowest effective dose for the shortest possible duration of treatment [54–61].

Neptune Krill Oil is a rich source of unique phospholipid carriers of omega-3 fatty acids, eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), esterified on antioxidants, as astaxanthin and a novel flavonoid. Phospholipids are important in protecting membranes from toxic injury and free radical attack [39]. The composition of phospholipids in Neptune Krill Oil appears to be optimal to offer such protection. The unraveling of the exact mechanism of action is a multifactorial project which is still ongoing. We speculate that it is based on the blockage of leukotriene formation by interfering at the level of the lipoxygenase pathways. The significantly dominant omega-3 to omega-6 ratio (15:1) in Neptune Krill Oil may partially explain the anti-inflammatory effects observed in this trial. The balance of polyunsaturated (essential) fatty acids in the body is critical for the maintenance of healthy cell membranes and hormone regulation. During the last decades, the American diet has shifted to much higher levels of omega-6 and less omega-3 fatty acid intake. Long-chain omega-6 such as arachidonic acid, predominating in the phospholipids of cell membranes can encourage the production of pro-inflammatory type-2 prostaglandins (PGE2), while omega-3 fatty acids promote the production of anti-inflammatory prostaglandins [1,2]. An additional factor is the naturally occurring astaxanthin in NKO™ which may also actively contribute in its anti-inflammatory potency. A recent study by Ohgami K. et al demonstrates that astaxanthin inhibits nitric oxide production through inhibiting the activity of inducible nitric oxide synthase (NOS), and

Table 5. Change in WOMAC Stiffness Scores/100 by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	7 Days	Mean	−20.29	−17.54	0.001
		Std Deviation	24.31	29.88	
		Median	−25.00	25.00	
		P-Value (Visit)	0.004	0.127	
	14 Days	Mean	−26.09	−0.70	0.018
		Std Deviation	27.05	20.55	
		Median	−31.25	1.00	
		P-Value (Visit)	0.002	0.820	
	30 Days	Mean	−39.13	4.21	0.023
		Std Deviation	27.67	26.74	
		Median	−31.25	12.50	
		P-Value (Visit)	0.003	0.879	

* 0 represents the best outcome and 4 the worst.

Table 6. WOMAC Functional Impairment Scores by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	Baseline	Mean	3.34	2.98	0.105
		Std Deviation	.91	.41	
		Median	3.41	3.12	
	7 Days	Mean	2.58	2.94	0.023
		Std Deviation	.58	.37	
		Median	2.82	3.00	
	14 Days	Mean	2.36	2.65	0.021
		Std Deviation	.31	.36	
		Median	2.56	2.63	
	30 Days	Mean	2.14	2.78	0.135
		Std Deviation	.68	.44	
		Median	2.66	2.91	
	P value (Within Groups)/Interaction		0.018	0.138	

* 0 represents the best outcome and 4 the worst.

Table 7. Change in WOMAC Functional Impairment Scores/100 by Group and Visit*

			Group		P value (Between Groups)
			NKO 300 mg/day	Placebo	
Visit	7 Days	Mean	−22.75	−1.34	0.008
		Std Deviation	10.59	5.86	
		Median	−2.53	1.55	
		P-Value (Visit)	0.005	0.750	
	14 Days	Mean	−29.34	−11.07	0.040
		Std Deviation	14.07	13.06	
		Median	−14.02	−6.15	
		P-Value (Visit)	0.016	0.094	
	30 Days	Mean	−35.93	−6.71	0.005
		Std Deviation	9.69	7.34	
		Median	−20.47	−3.11	
		P-Value (Visit)	0.08	0.269	

* 0 represents the best outcome and 4 the worst.

production of PGE2 and TNF-. This study suggests that astaxanthin may have an anti-inflammatory effect and may be a promising agent for the treatment of inflammation [43].

The present study confirms the results of previous research demonstrating the anti-inflammatory effects of EPA and DHA and of a dominant omega-3 versus omega-6 ratio [30–38,40,44–53]. Simopoulos has shown that omega-3 fatty acids lower CRP more so than any other nutrient, which accounts for decreasing the risk for coronary heart disease [45]. Human and animal studies have provided evidence that dietary intake of omega-3 fatty acids modifies inflammatory and immune reactions. This makes making omega-3 fatty acids potential therapeutic agents for inflammatory diseases [30–38,40,44–53].

A possible explanation for the increase of CRP observed in the placebo group is the interruption of all anti-inflammatory regimens one week prior and for the duration of the trial. Since the patients enrolled suffered from a chronic inflammatory condition with chronically high CRP, the cessation of all anti-inflammatory treatment may have triggered the increased production of CRP.

The significant reduction of pain shown in the WOMAC pain score is also demonstrated the significantly lower consumption of NSAIDs by the group of patients treated with NKO™. This finding becomes even more significant if we consider the nephrotoxicity of NSAIDs mainly among patients with chronic inflammatory diseases.

The results of the present study validate the potent anti-inflammatory properties of NKO™ and reinforce the potential mechanism of action. The CRP reduction induced by NKO™ demonstrates that NKO™ is a safe and effective alternative for the treatment of inflammation, particularly with all the recently proven adverse events of the most widely used NSAIDs. Furthermore, this study demonstrates a significant improvement in all 3 WOMAC scores among the 30 and 10 patients on NKO™ as compared to the 26 and 12 patients on placebo who were diagnosed with osteoarthritis and rheumatoid arthritis. No adverse events were reported making NKO™ safe for human consumption.

CONCLUSION

The results of the present study indicate that NKO™ at a daily dose of 300 mg may within a short time to reaction (7–14 days) significantly inhibit inflammation by reducing CRP as well as significantly alleviate symptoms caused by osteoarthritis and rheumatoid arthritis. Further research is required to better understand the mechanism of action and to compare the effects of NKO with other anti-inflammatory agents presently used as standard care.

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Received April 30, 2005; revision accepted September 12, 2006.