

# First Study of Infliximab Treatment in Patients with Chronic Obstructive Pulmonary Disease

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**Rationale:** Tumor necrosis factor- $\alpha$  is believed to be important in the induction and maintenance of airway inflammation in chronic obstructive pulmonary disease. **Objectives:** We aimed to evaluate the effect of the anti-tumor necrosis factor- $\alpha$  drug infliximab in patients with chronic obstructive pulmonary disease, with percentage of sputum neutrophils as the primary endpoint. **Methods:** We performed an exploratory single-center, double-blind, placebo-controlled, randomized, phase 2 trial in which 22 current smokers with mild-to-moderate chronic obstructive pulmonary disease participated. Fourteen patients received three infusions of infliximab (5 mg/kg) at Weeks 0, 2, and 6, and eight patients received placebo infusions. Sputum samples, respiratory symptoms, quality of life, exhaled nitric oxide, lung function parameters, bronchial hyper-responsiveness, resting energy expenditure, and side effects were evaluated. **Measurements and Main Results:** This study did not show a positive short-term effect of infliximab on airway inflammation, lung function, resting energy expenditure, or quality of life. Exhaled nitric oxide increased significantly at Day 2, Week 6, and Week 8 in patients receiving infliximab compared with those receiving placebo. Eight patients in the infliximab group (vs. none in the placebo group) reported increased coughing, but no serious adverse events or increase in respiratory infections were reported during 9 weeks of follow-up. **Conclusions:** In this short-term study, no clinically beneficial effects of infliximab were observed, and there were no significant safety issues. Definite conclusions concerning the effectiveness of infliximab treatment in chronic obstructive pulmonary disease await additional studies, including those with a larger number of patients with more advanced disease.

**Keywords:** chronic obstructive pulmonary disease; inflammation; tumor necrosis factor- $\alpha$

Chronic obstructive pulmonary disease (COPD) is an inflammatory lung disease resulting in progressive lung function loss. It is one of the leading causes of morbidity and mortality worldwide, and its prevalence is still rising (2). Smoking cessation at an early stage of the disease is the most important intervention to slow down lung functional loss, but success rates are low (3), and airway inflammation appears to persist (4). Unfortunately, no antiinflammatory agents are currently available that effectively reduce the underlying airway inflammation and/or stop this progressive decline in lung function (5).

Airway inflammation in COPD is characterized by the presence of mononuclear cells, neutrophils, and their mediators. The cytokine tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is believed to play a

central role in the pathophysiology of COPD (6). TNF- $\alpha$  enhances neutrophil chemotaxis and migration by inducing the expression of chemokine interleukin 8 (IL-8) and upregulating endothelial adhesion molecules (7, 8). *In vivo*, elevated levels of TNF- $\alpha$  have been demonstrated in peripheral blood and sputum of patients with stable COPD (9, 10), and a polymorphism of the promoter region of the TNF- $\alpha$  gene has been implicated in the occurrence, severity, and mortality risk of COPD (11–13).

Nutritional depletion is present in 20% of patients with COPD (14) and is associated with an increase in daily energy expenditure and in levels of inflammatory mediators (15). TNF- $\alpha$  seems to be especially important among these inflammatory mediators because it correlates with increased energy expenditure. Furthermore, increased serum TNF- $\alpha$  levels are found in a subgroup of patients with nutritional depletion (9, 16, 17).

Infliximab is a chimeric monoclonal antibody that binds with high affinity and specificity to human soluble and membrane-bound TNF- $\alpha$  and neutralizes its biologic activity (18, 19). Infliximab has been shown to improve clinical and inflammatory parameters in patients with Crohn's disease and rheumatoid arthritis (20–23). We designed a randomized, double-blind, placebo-controlled, proof-of-principle phase 2 study to evaluate whether short-term treatment with infliximab inhibits airway inflammation in patients with stable COPD, with percentage of sputum neutrophils being the primary endpoint. Secondary objectives of the study included evaluation of the effect of infliximab on respiratory symptoms, quality-of-life indicators, and lung function parameters. Another secondary objective was the evaluation of safety and tolerability of short-term infliximab therapy in COPD. Tertiary objectives included evaluation of infliximab treatment's effects on resting energy expenditure (REE) levels, and on exhaled nitric oxide (eNO) values. Some of the results of these studies have been previously reported in the form of an abstract (1).

## METHODS

### Design of the Study

We performed a single-center, randomized, double-blind, placebo-controlled pilot study. Patients with COPD were randomized to treatment with infliximab (Remicade; Centocor, Malvern, PA) at 5 mg/kg (14 patients) or with placebo (8 patients), with each treatment to be administered by infusion at Weeks 0, 2, and 6. A minimization method was used to provide groups balanced for age ( $\leq 60$  or  $> 60$  years), FEV<sub>1</sub> ( $\leq 60$  or  $> 60\%$ ), and number of exacerbations per year ( $\leq 2$  or  $> 2$ ). Sputum samples, spirometry, diffusion capacity, eNO, and REE were evaluated at Day 1 (baseline) and Day 2, and at Weeks 2, 6, and 8. The Clinical COPD Questionnaire (CCQ) (24) was evaluated at Day 1, and at Weeks 2, 6, and 8. The St. George Respiratory Questionnaire (SGRQ; assessing quality of life) and the concentration of adenosine 5'-monophosphate causing FEV<sub>1</sub> to drop by 20% (PC<sub>20</sub> AMP) were measured at baseline and subsequently at Weeks 8 and 9, respectively. Safety evaluations are described in the online supplement.

### Subjects

Twenty-two patients with symptomatic COPD were enrolled, all with a postbronchodilator value FEV<sub>1</sub>/FVC ratio below 70% and FEV<sub>1</sub> greater

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TABLE 1. PATIENT CHARACTERISTICS

	Infliximab	Placebo
No. patients	14	8
Sex, M/F	13/1	5/3
Age, yr	56.6 ± 6.1	54.8 ± 7.4
Smoked, pack-yr	60.6 ± 40.3	53.8 ± 35.6
Smoked, cigarettes/d	23.8 ± 15.3	28.8 ± 15.9
BMI, kg/m <sup>2</sup>	24.0 ± 3.9	26.8 ± 3.0
FEV <sub>1</sub> , % pred	80.5 ± 9.2	86.1 ± 15.9
FEV <sub>1</sub> /FVC, %	62.1 ± 4.7	60.8 ± 9.3
PC <sub>20</sub> AMP, mg/ml	12.2 (1.1–640)	20.4 (3.7–640)
CCQ, total	1.2 ± 0.6	1.7 ± 0.7
SGRQ, total	34.1 ± 12.4	28.0 ± 14.5

Definition of abbreviations: BMI = body mass index; CCQ = Clinical COPD Questionnaire; M/F = male/female; PC<sub>20</sub> AMP = the concentration of adenosine 5'-monophosphate causing FEV<sub>1</sub> to drop by 20%; SGRQ = St. George Respiratory Questionnaire.

Values are presented as means ± SD or as geometric means (ranges). There were no differences between the two groups.

than 1.5 L. All patients were current smokers (> 5 cigarettes/day, > 10 pack-years). Exclusion criteria are presented in the online supplement. The medical ethics committee of our institute approved the study. Written, informed consent was obtained from all patients.

### Pulmonary Function Tests, REE, and eNO

FEV<sub>1</sub> and FVC were measured according to the guidelines of the European Respiratory Society (25). Diffusion capacity for carbon monoxide (expressed by transfer factor for carbon monoxide [TL<sub>CO</sub>] and TL<sub>CO</sub> divided by the alveolar volume [K<sub>CO</sub>]) was measured using the single breath-holding method, according to international guidelines (25). Airway hyperresponsiveness to adenosine 5'-monophosphate was determined using standardized methods (26). REE was measured by an open-circuit, indirect calorimetry system using a ventilated hood system (Oxyconbeta; Jaeger, Wurzburg, Germany), as described previously (27). eNO levels were determined by a chemiluminescence analyzer (CLD 700 AL; Ecophysics, Durnten, Switzerland) using the American Thoracic Society guidelines (28).

### Sputum Induction and Processing

Sputum was induced according to a modified standard technique (29) using 4.5% hypertonic saline. Sputum was processed within 120 minutes by a modified method of Rutgers and colleagues (29).

IL-6 and IL-8 were measured in sputum supernatant by commercial ELISA (Sanquin, Amsterdam, the Netherlands). Neutrophil elastase activity was measured by chromogenic substrate assay (*N*-methoxysuccinyl-ala-ala-pro-val-p-nitroanilide; Sigma, Poole, UK) (30).

### Statistical Analyses

Clinical characteristics are presented as means (± SD) unless stated otherwise. All effect parameters are presented as medians with ranges. Differences in changes from baseline between the two treatment arms were tested with a Mann-Whitney test. A *p* value of less than 0.05 was considered statistically significant.

### RESULTS

No significant differences in baseline characteristics were observed between the infliximab and placebo treatment groups (Tables 1 and 2). Infliximab treatment was not associated with significant changes in the absolute number or percentage of inflammatory cells in sputum compared with that in placebo (Table 3). In particular, infliximab treatment did not affect the primary endpoint of the study, the percentage of sputum neutrophils. In addition, changes in IL-8 and IL-6 levels did not differ between infliximab and placebo treatment groups (Table 3). Neutrophil elastase could not be detected in all sputum samples.

Changes in FEV<sub>1</sub>, FEV<sub>1</sub>/FVC (data not shown), and K<sub>CO</sub>/VA did not differ between the infliximab and placebo treatment groups (Table 2). The median change in eNO, however, was significantly higher at Day 2, Week 6, and Week 8 with infliximab treatment compared with placebo. Changes in REE and bronchial hyperresponsiveness to adenosine 5'-monophosphate did not differ between the infliximab and placebo treatment groups (Table 2), nor did respiratory symptoms and quality-of-life indicators (data not shown).

TABLE 2. LUNG FUNCTION PARAMETERS

	Baseline	Day 2	Week 2	Week 6	Week 8
Placebo					
eNO, ppb	7.4 4.7–17.8	5.1 3.4–11.1	6.2 3.3–17.1	4.4 2.7–9.1	5.9 2.6–11.6
FEV <sub>1</sub> , % pred	76.6 52.9–100.9	75.2 49.3–103.6	76.4 50.7–98.6	80.9 53.0–99.5	79.4 57.0–101.0
TL <sub>CO</sub> /VA, % pred	76.1 48.0–113.0	77.2 49.3–118.8	76.9 54.0–115.6	77.8 50.7–118.8	80.8 45.3–114.9
REE, % pred	114.1 89.3–125.2	109.4 81.5–125.5	107.0 86.9–121.1	110.6 100.9–124.7	101.1 93.7–122.0
PC <sub>20</sub> AMP, mg/ml*	20.4 3.7–640	ND	ND	ND	10.3 0.3–84.6 <sup>‡</sup>
Infliximab Treatment					
eNO, ppb	7.7 3.1–26.1	9.4 <sup>†</sup> 3.8–28.7	8.7 3.8–37.5	9.6 <sup>‡</sup> 2.8–47.5	9.7 <sup>‡</sup> 6.0–29.1
FEV <sub>1</sub> , % pred	77.9 53.6–96.4	79.2 49.3–93.0	77.7 55.5–100.6	79.6 42.6–96.0	76.8 46.4–93.1
TL <sub>CO</sub> /VA, % pred	83.7 57.6–108.6	83.0 54.2–107.8	85.8 56.9–120.1	81.9 53.5–107.9	82.3 51.4–102.9
REE, % pred	102.4 83.3–116.0	101.9 88.6–129.5	104.5 95.7–121.3	103.8 91.3–124.0	103.5 90.4–136.6
PC <sub>20</sub> AMP, mg/ml*	12.2 1.13–640	ND	ND	ND	11.4 0.9–640 <sup>‡</sup>

Definition of abbreviations: eNO = exhaled nitric oxide; ND = not done; REE = resting energy expenditure; TL<sub>CO</sub>/VA = diffusion capacity for carbon monoxide corrected for the alveolar volume.

Values are presented as medians or geometric means (\*) with ranges.

<sup>†</sup> The change from baseline is significantly different with infliximab compared with placebo, *p* < 0.05.

<sup>‡</sup> PC<sub>20</sub> AMP is measured at Week 9.

TABLE 3. SPUTUM INFLAMMATORY PARAMETERS

	Baseline	Day 2	Week 2	Week 6	Week 8
Placebo					
	7.7	5.8	8.0	7.1	7.1
Sputum weight, g	1.6–13.5	2.1–14.5	1.2–12.1	2.8–12.7	1.0–10.4
	2.2	2.5	4.3	3.8	1.9
Total cells, 10 <sup>6</sup> /ml	1.1–14.8	0.6–12.5	1.9–5.7	0.6–5.3	0.6–12.0
	26.7	35.7	27.0	26.8	37.8
Macrophages, %	21.0–41.2	19.0–62.2	13.3–35.2	9.8–45.5	13.5–47.3
	68.3	61.4	66.7	68.7	57.3
Neutrophils, %	56.8–77.3	36.5–74.3	49.0–73.8	50.5–84.2	49.7–82.0
	0.7	0.8	1.0	1.2	1.0
Eosinophils, %	0.3–1.8	0.0–1.7	0.5–1.80	0.3–2.5	0.5–2.0
	0.3	0.7	0.7	0.7	0.5
Lymphocytes, %	0.2–2.0	0.0–0.8	0.0–5.2	0.0–1.5	0.0–1.8
	2,470	2,317	2,071	1,756	1,811
IL-8, ng/ml	1,117–20,749	1,485–14,286	1,224–28,409	697–29,015	932–27,737
	146	106	103	87.4	80.4
IL-6, ng/ml	16.3–200	12.4–260	20.1–584	10.7–428	9.6–596
Infliximab Treatment					
	6.0	7.5	6.2	7.7	8.0
Sputum weight, g	1.3–17.0	1.9–16.3	3.7–14.0	2.3–12.6	1.6–14.8
	3.8	3.5	4.6	5.2	5.2
Total cells, 10 <sup>6</sup> /ml	0.2–24.5	0.4–11.5	0.5–23.2	0.7–29.2	1.1–48.9
	26.3	36.0	26.8	27.1	27.0
Macrophages, %	16.3–48.7	10.5–56.5	6.5–55.5	10.2–42.2	4.8–52.3
	66.5	49.7	62.9	62.0	65.7
Neutrophils, %	48.7–80.2	31.3–81.3	24.3–90.3	47.5–85.0	18.3–92.0
	1.3	1.5	1.8	1.4	1.5
Eosinophils, %	0.3–16.0	0.3–18.5	0.2–23.7	0.0–18.7	0.0–18.7
	0.5	0.5	0.5	0.7	0.8
Lymphocytes, %	0.2–4.8	0.0–1.3	0.0–2.3	0.0–4.5	0.3–3.0
	7,199	4,102	5,334	4,613	3,624
IL-8, ng/ml	954–16,526	1,197–13,430	1,040–20,490	439–32,280	1,264–32,412
	223	130	179	164	123
IL-6, ng/ml	18.2–1,181	12.5–369	3.8–1,595	7.7–1,926	9.8–1,181

Definition of abbreviation: IL = interleukin.

Values are presented as medians with ranges. There were no significant differences between the two groups.

No serious adverse events were reported for either treatment group during the 9-week follow-up period. All three infusions were generally well tolerated; one subject who received infliximab withdrew from the study after the first infusion due to flulike symptoms without other evidence of serious disease or any abnormalities shown by physical examination or laboratory values. The most frequently reported adverse events are shown in Table 4. Eight patients in the infliximab group reported in-

creased cough, an incidence that was significantly greater than in the placebo group. Patients did not report more respiratory tract infections after infliximab treatment than those who received placebo, and no bronchospasm occurred. One patient had an asymptomatic increase in antinuclear antibody titer after the third infusion of infliximab; otherwise, no significant laboratory changes were observed during the study period.

## DISCUSSION

The proinflammatory cytokine TNF- $\alpha$  is believed to play a central role in the induction and maintenance of airway inflammation in COPD. In the present study, however, administration of the anti-TNF- $\alpha$  drug infliximab to a group of patients with well-characterized COPD did not result in improvement of any of several clinical parameters, including FEV<sub>1</sub>, CCQ, and quality-of-life indices compared with a control group receiving placebo infusions. Airway inflammation, including percentage of sputum neutrophils and level of IL-8 and REE, also did not change.

One explanation for the lack of response may be that the degree of severity of COPD in our patients was too low to show a significant response. It has been shown that patients with lower FEV<sub>1</sub> values have a greater degree of airway inflammation, as reflected by higher levels of sputum neutrophils and IL-8 (10, 31). Because patients with more severe disease are more prone to respiratory tract infections and bronchospasm, we chose to evaluate patients with only mild-to-moderate COPD. We had to take into account the safety and tolerability of infliximab in

TABLE 4. ADVERSE EVENTS

	Infliximab, No. Patients (n = 14)	Placebo, No. Patients (n = 8)
Coughing	8*	0
Sputum production	5	1
Dyspnea	4	2
Irritated bowel	3	1
Nose cold	3	0
Respiratory tract infection	3	3
Dizziness	3	0
Diarrhea	2	2
Chest pain	2	1
Myalgia	2	0

At each visit, the following question was asked to evaluate adverse events: "Did you have any complaints (especially fever, or extra or new symptoms) since the last visit?"

\*p < 0.05 compared with placebo.

patients with COPD because this was the first study to evaluate infliximab in the treatment of this condition. Thus, infliximab could possibly prove effective in patients with more severe COPD. Another explanation for the observed lack of clinical response may be that TNF- $\alpha$  is not a cytokine that plays a central role in the pathophysiology of COPD, and the observed increase in TNF- $\alpha$  is merely an epiphenomenon. There is a wide pleiotropy and functional redundancy among cytokines, with each function potentially mediated by more than one cytokine (32). Actions attributable to TNF- $\alpha$  may in fact be exerted by other cytokines, such as IL-1 $\beta$ , IL-8, and IL-6. Other conditions, such as sepsis and heart failure, which are associated with increases in a variety of proinflammatory cytokines, are not mitigated in response to anti-TNF- $\alpha$  therapy as well (33, 34).

Lack in observed clinical response could also be related to the study design. All of our patients were current smokers, and it has been shown previously that, at least in patients with Crohn's disease, smoking has a strong adverse effect on the rate and duration of response to infliximab (35). It might also be that the dose of infliximab used was too low, that the duration of our treatment regimen was too short to induce changes in some of our parameters, and/or that follow-up may have been too short to measure significant changes. The same dose of infliximab, however, has produced antiinflammatory and clinical effects within a few weeks in patients with rheumatoid arthritis and Crohn's disease (20, 23). In addition, our study was designed as a pilot study, with a small number of patients and low power to detect changes in our parameters. Finally, it is possible that the sputum parameters we measured may correlate only moderately with inflammatory parameters in lung tissue itself (29).

eNO levels were unexpectedly higher after infliximab treatment than was observed after placebo treatment. Although the change in eNO reached statistical significance, it is of questionable clinical importance, because the median change in eNO was only 2 ppb. Interestingly, an increase in eNO is also observed after smoking cessation (36), suggesting a similar, possibly beneficial mechanism of infliximab treatment in COPD. The significance of this finding is only speculative but could merit further investigation.

Because a higher serum TNF- $\alpha$  has been associated with increased energy expenditure in COPD (16), we wanted to see if infliximab would attenuate the REE in our subjects; however, no significant effect was demonstrated. This could be explained by the fact that the REE values for our patients were generally within the normal range at baseline.

No serious adverse events were reported within the 9 weeks of follow-up. Patients did not report more respiratory tract infections after infliximab treatment than those who received placebo, and no bronchospasm occurred in either treatment group. Increased cough was reported by eight patients in the infliximab group, but by none of the patients receiving placebo. Coughing is a frequently reported adverse event after infliximab treatment when given for other diseases, and might be more pronounced in our patient group (37). However, this finding was not supported by the results obtained from the respiratory symptom domains in the CCQ and SGRQ.

In conclusion, our study did not show a short-term improvement in clinical or inflammatory parameters from infliximab treatment in patients with mild-to-moderate COPD, although patients so treated did demonstrate an increase in eNO, a finding of uncertain clinical relevance. Importantly, no severe adverse events occurred and there was no reported increase in the prevalence of respiratory tract infections in 9 weeks of the study. Of patients with COPD who received infliximab, 8 of 14 reported increased coughing, yet this was not reflected in answers to questions about symptoms in the CCQ and SGRQ. Definitive

conclusions as to the effectiveness of anti-TNF- $\alpha$  treatment in COPD must await additional studies involving patients with COPD with more severe disease, in larger cohorts and with longer follow-up periods.

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