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CLINICAL REPORT

Targeting Oxidative Injury and Cytokines' Activity in the Treatment with Tumor Necrosis Factor- α Antibody for Complex Regional Pain Syndrome 1

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Abstract: Cytokines and oxygen free radicals have been implicated in the potential pathogenic development of complex regional pain syndrome (CRPS). We aimed to analyze the relationship between clinical status, circulating levels of cytokines, and markers of oxidative damage during the treatment with anti-TNF α antibodies. The patient chosen for treatment had not had improvement through a number of conventional therapies and fulfilled the current diagnostic criteria for CRPS-1. We investigated the clinical variables before and after systemic administration of 1.4 mg/kg TNF antibody (infliximab), repeated after 1 month in a dose of 3 mg/kg. Blood samples were collected before and after anti-TNF α

administration, and plasma was analyzed for 8-isoprostane-prostaglandin F $_{2\alpha}$ (8-iso-PGF $_{2\alpha}$, a marker of oxidative injury) and cytokines (TNF- α , IL-4, IL-6, IL-7, IL-8, IL-10, IL-17A). Plasma concentrations of 8-iso-PGF $_{2\alpha}$ were measured with radioimmunoassay (RIA), and the kinetics of cytokines were detected in plasma by antibody-based proximity ligation (PLA). Pathologically high levels of 8-iso-PGF $_{2\alpha}$ were found in the patient. Immediately after each administration of infliximab, the levels of 8-iso-PGF $_{2\alpha}$ decreased. Although the patient showed an improvement of the cutaneous dystrophic symptoms and diminished pain associated with these lesions, the levels of circulating TNF α increased after the administration of anti-TNF α antibodies. In a patient with CRPS-1 treated with anti-TNF α antibodies, we report increased levels of circulating TNF α and a temporary mitigation of oxidative stress as measured by plasma F $_2$ -isoprostane. This case report provides evidence supporting monitoring of the oxidative stress biomarkers during treatment with anti-TNF α antibodies in CRPS 1. ■

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CASE DESCRIPTION

A 47-year-old, 77-kg woman was referred to our pain center with a 3-year history of pain in her left shoulder that progressed in intensity. Six months after the initial symptoms, her entire left and right arms became involved. Her illness began after a fall, causing a subluxation of her left shoulder that was treated conservatively. Radiographs of her left shoulder and both arms revealed no fracture or degenerative changes. Her pain was rated as 8–9 on a 0–10 NRS scale and described as a shooting pain and burning sensation in both arms. It was associated with paresthesias, autonomic dysfunction consisting of edema, and color and temperature differences between the arms. Cervical MRI was normal. Sensory evoked potentials (SEP), neurography, and electromyography (EMG) were normal. Several months after the onset of pain, trials of pregabalin, gabapentin, amitriptyline, duloxetine, opioids, and baclofen were tried without success. Edema with associated weakness, increased sweating in the upper extremities, temperature changes, mechanical allodynia, cutaneous dystrophic, and atrophic changes were present. Ulcerating skin lesions of the fingertips with reduced nail growth and loss of nails were noted on clinical examination. Due to the pain in her fingertips, the patient covered her nails with medical tape for protection. The pain from the lesions in her hands (Figure 1) was self-reported as 86 on a visual analog scale (VAS 0–100). The symptoms were interpreted as secondary to CRPS, and the patient was referred to our multidisciplinary pain center for treatment. Sensory examination

Figure 1. The patient presented ulcerating skin lesions in the fingertips with reduced nail growth and loss of nails before the first treatment with anti-TNF α .

revealed mechanical allodynia and hyperesthesia. The treatment included a combination of physical and occupational therapy and pharmacologic management including opioids, a long-acting morphine (Dolcontin[®], Pfizer AB, Sollentuna, Sweden) 30 mg twice daily, a short-acting oxycodone (OxyNorm[®], Mundipharma AB, Ghotenburg, Sweden) 5 mg as needed, and lidocaine patches 5% (Versatis[®] Grünenthal, Solna, Sweden) on her fingertips. The patient fulfilled the diagnostic criteria for CRPS-1.¹ All treatments initiated failed to improve the patient's condition, so we decided to try TNF α antibodies. We documented the clinical variables (pain, edema, color, temperature changes, cutaneous changes, mechanical, and cold allodynia) before and after systemic administration of 1.4 mg/kg TNF α antibody, infliximab (Remicade[®]), repeated after 1 month in a dose of 3 mg/kg. Because there is evidence that treatment with a TNF α antagonist increases the risk of reactivating tuberculosis² and screening for tuberculosis infection is recommended before treatment is initiated,³ a chest radiograph and tuberculosis screening test were performed before treatment. The tests were negative. The patient's clinical signs were recorded, and qualitative sensory testing measurements were taken the day before, immediately after infusion and 1 week after treatments. A health survey (SF-36)^{4,5} was documented before and after treatments. Blood was obtained for analysis including whole blood cell counts, electrolyte studies, renal and liver functions tests, blood glucose, anti-CCP (anti-cyclic citrullinated peptide antibody) and C-reactive protein (CRP) levels, F₂-isoprostanes, and cytokines. Plasma concentrations of 8-iso-prostaglandin F_{2 α} (8-iso-PGF_{2 α}), a major F₂-isoprostane (indicator of in vivo oxidative injury), were measured according to a specific and validated radioimmunoassay (RIA) method at our laboratory, as previously described by Basu.^{6,7} The detection limit for free 8-iso-PGF_{2 α} is 23 pmol/L with mean plasma levels in healthy humans about 79 pmol/L. Cytokines (TNF- α , IL-4, IL-6, IL-7, IL-8, IL-10, IL-17A) were detected in plasma by antibody-based proximity ligation 10 minutes before the infusion of infliximab, 2 hours after treatment, 1 month after the first infusion, and 2 hours after the second infusion. Proximity ligation assay has been used to develop homogeneous immunoassays to detect cytokines in the sub-pg/mL range.^{8,9}

Clinical Status

Several hours after the first administration of infliximab, the patient reported a decrease in the pain in her

fingertips, a decrease in the static and dynamic cutaneous allodynia, and less edema in her arms. The lesions of the patient's fingertips looked considerably better a month after the treatment (Figure 2) but the same symptoms returned in her arms. No effect on the mechanical allodynia was observed after the second administration of infliximab. Long-term follow-up of the patient indicated a healing of the ulcerations in the fingertips, but the pain, edema, and temperature disturbances continued in her arms.

Questionnaire Data

Table 1 provides the scores from a health survey (SF-36) that yields an 8-scale profile of functional health and well-being scores as well as a physical and mental health summary.^{4,5} The results from the SF-36 show her disease was very disabling before and after treatment as evidenced by the low scores in functional health, well-being, and physical and mental health (limited capacity in performing physical activities, limitations in social activities, severe pain, psychological distress, fatigue, and no energy). There was a slight increase in the physical function score after the first treatment and an increase in mental health and social functioning scores after each administration 1 week after both treatments.

4.2.4 F₂-Isoprostanes (In Vivo Oxidative Stress Indicator)

Plasma levels of free 8-iso-PGF_{2α} were found to be 111 pmol/L, which is over the mean plasma levels in

healthy human subjects (normal < 79 pmol/L) before treatment with infliximab and this marker decreased to 64.4 pmol/L, after the treatment. One month after the treatment, the plasma levels were found to be 203 pmol/L that decreased to 149.8 pmol/L (Table 1) after the second treatment with infliximab.

Serum Cytokines' Levels

A significant increase in circulating TNF-α was observed immediately and 1 month after infliximab administration (Figure 3). This was statistically significant in comparison with baseline. One month after administration of infliximab, a small increase was observed also in IL-4, IL-6, and IL-17 but no effects on other cytokines.

Laboratory Investigations

Except for a significant decrease in the GFR from 80 to 45, all other laboratory investigations were normal before and after the administration of anti-TNFα (Table 2).

DISCUSSION

Other possible diagnoses in the differential, including rheumatoid arthritis and other inflammatory arthropathies, were excluded in our patient by assays of nonspecific inflammatory markers, including C-reactive protein (CRP), leukocyte count (WBC), sedimentation rate (SR), and anti-citrullinated protein antibodies (ACPA), which is highly predictive of and specific for RA¹⁰ and a useful marker for systemic sclerosis. The use of TNF-α inhibitors has no negative effect on renal function¹¹; thus, the decrease in GFR was not related to the treatment. CRPS has been assumed by some investigators to be a neurological inflammation with involvement of an active immune system that could generate neuropeptides, cytokines, and eicosanoids, which could secondarily generate mediators of inflammation.^{12,13}

Although no specific laboratory test has been discovered to monitor therapy, treatment with scavenger therapy is widely applied in CRPS-1,^{14,15} as well as prophylactic therapy after fractures to prevent the occurrence of CRPS-1.^{16,17} In a randomized controlled trial, topical treatment with 50% dimethyl sulfoxide (DMSO) led to an improvement of pain and inflammatory signs in CRPS.¹⁵ Two randomized clinical trials indicated a prophylactic effect of vitamin C on the development of CRPS after wrist fracture.^{16,17} A

Figure 2. One month after the treatment, the ulcerating skin lesions in the fingertips disappeared and the nails looked considerably better.

Table 1. Numerical Rating Scale, Oxidative Injury Biomarker (8-iso-PGF2 α), and Physical and Mental Health Scale Scores (SF-36), Before and After the 1st and 2nd Treatment

Measures	Time	Before 1st treatment (mean)	After the 1st treatment (mean)	Before the 2nd treatment (mean)	After the 2nd treatment (mean)
Continuing pain NR (0–10)	9.00 AM	9.0	8.3	9.0	8.3
	3 PM	8.6	7.8	8.6	7.9
	9.00 PM	7.6	7.3	8.7	8.5
Oxidative stress SF-36	8-iso-PGF2 α (pmol/mL) <i>N</i> = 79	111	64.4	203	149.8
	PF (physical function) <i>N</i> = 87.5	10	20	20	20
	RP (physical role function) <i>N</i> = 83.2	0	0	0	0
	BP (body pain) <i>N</i> = 74.8	0	0	11	13
	GH (general health) <i>N</i> = 75.8	8	10	6	8
	VT (vitality) <i>N</i> = 68.8	0	0	0	0
	SF (social function) <i>N</i> = 88.1	0	25	0	23
	EF (emotional function) <i>N</i> = 85.7	0	0	0	0
	MH (mental health) <i>N</i> = 80.9	8	30	9	28

Figure 3. Visual analog scale (VAS) (a), 8-iso-PGF2 α (b), levels of circulating TNF α (c). (a) Visual analog scale 1 week before the 1st infliximab administration as mean at 9.00 AM, 3.00 PM, and 9.00 PM (1); 1 week after the 1st anti-TNF- α administration (2); 1 week before the 2nd administration of anti-TNF- α (3), and 1 week after the 2nd administration of anti-TNF- α (4). (b) Plasma levels of 8-isoprostane-prostaglandin F2 α (8-iso-PGF2 α) in pmol/L, at baseline (1), two hours after the 1st infliximab administration (2); after 1 month and before the 2nd administration of (3); two hours after the 2nd administration of infliximab (4). (c) Proximity ligation assay demonstrates the levels of circulating TNF- α in serum at baseline (1) two hours after the 1st infliximab administration (2); after 1 month (3); two hours after the 2nd administration of infliximab (4).

positive effect of N-acetylcysteine has been reported to be equally effective as DMSO treatment in CRPS-1.¹⁴ The antioxidants appear to be effective only in association with analgesics and physical and occupational therapy.¹⁴

F₂-isoprostanes are reliable, the most specific biomarkers of lipid peroxidation, and are useful in vivo indicators of oxidative stress and to assess the oxidative response to various drugs, antioxidants, or dietary interventions.¹⁸ We confirmed the presence of oxidative

Table 2. Laboratory Tests Before Administration of Anti-TNF α and at Follow-up at 1 Month After Administration of Anti-TNF α

Laboratory	Normal levels	Before the 1st anti-TNF α administration	One month after anti-TNF α administration
CRP	mg/L	0.63	5
SR	< 10 mm/h	7	7
Hb	123–157 g/L	135	128
Red blood cells	4.0–5.2 $\times 10^{12}$ /L	4.29	4.45
MCV	80–100 fl	92.2	86.8
MCH	27–34 pg	32	29
White blood cells	4–10 $\times 10^9$ /L	6.3	6.5
Platelet count	130–400 $\times 10^9$ /L	292	431
Vitamin B12	74–516 pmol/L	1,221	1,094
Folic (folate)	7–36 nmol/L	3.8	6
INR	0.9–1.2	0.9	1.1
Sodium (serum)	135–145 mmol/L	138	142
Potassium (serum)	3.5–4.5 mmol/L	4.2	4.3
Creatinine (serum)	50–90 μ mol/L	71	72
GFR	> 80 mL/min/1.73	80	45
Albumin (serum)	35–50 g/L	43	34
Calcium (serum)	2.18–2.58 mmol/L	2.28	2.27
Alanine	3–36 U/L	26	28
Aspartate	0–35 U/L	5	25
Gamma-glutamyl transferase	10–30 U/L	27	25
ACPA	Negative < 20 EU	6	8

CRP, C-reactive protein; SR, sedimentation rate; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; ACPA, anti-citrullinated protein antibodies; INR, international normalized ratio; GFR, glomerular filtration rate.

stress reflected by higher levels of a major F₂-isoprostane (8-iso-PGF_{2 α}) in this patient. Our major finding is antibody neutralization of TNF- α in CRPS-1 reduces, but does not eliminate, the oxidative injury as measured by plasma 8-iso-PGF_{2 α} . Although its half-life has been found to be ~16 min in humans, 8-iso-PGF_{2 α} is a recommended biomarker of oxidative stress over time.¹⁸ One may speculate that the rise of 8-iso-PGF_{2 α} 1-month after the first administration correlated with the symptoms and pain that later returned in our patient's arms. The modest decrease in 8-iso-PGF_{2 α} after the second administration correlated with the clinical status where no other clinical improvement was seen except for less pain in her fingertips.

Anti-TNF- α antibodies have antioxidant properties,^{19,20} explaining the decrease in 8-iso-PGF_{2 α} after the treatment with anti-TNF- α antibodies. Several studies support the role of free radicals in the pathophysiology of CRPS-1^{21–23} and are based on the assumption that CRPS-1 is induced by an exaggerated inflammatory response to tissue injury, mediated by an excessive production of toxic oxygen radicals.²⁴ Codre et al. demonstrated that free radicals are involved in the pathogenesis of the signs of CRPS-1 in a rat model of CRPS.²⁵ Increased oxidative markers such as MDA

(malondialdehyde) and superoxide dismutase (SOD) activity have been observed in both saliva and serum of patients with CRPS-1²⁶ as indirect markers of oxidative stress. Recently, oxidative stress and nuclear factor erythroid 2-related factor (Nrf2) activation were proposed as key developments in the pathogenesis of CRPS.^{27,28} It has been speculated that functional polymorphisms of Nrf2, which is a regulator of the transcription of multiple antioxidants, are responsible for impaired endogenous antioxidant “defense” system, increasing the susceptibility to oxidative stress and the development of CRPS.^{27,28}

Accumulating evidence from animal studies^{29,30} and clinical studies in humans³¹ has demonstrated that TNF- α plays an important role in the development of cutaneous allodynia in CRPS. Increased serum levels of TNF- α receptor type 1 have shown to be correlated with the presence of mechanical allodynia in CRPS.³¹ However, other studies show no such changes.³² Increased levels of IL-6 and TNF- α were detected locally in the blister fluid of the affected limb³³ and in spinal fluid³⁴ of the patients suffering from CRPS. The local levels may be more relevant in the pathogenesis of CRPS³⁵ as seen in the peripheral elevations of TNF levels in fluids obtained from experimental blisters on the affected, but not the contralateral limbs of patients with CRPS.³³ Perhaps, skin blister levels of TNF from the arms in comparison with data from the unaffected limb would have been a better test in monitoring the treatment with anti-TNF α in this patient.

Although elevated levels of pro-inflammatory cytokines (TNF- α , IL-2) and reduced levels of anti-inflammatory cytokines (IL-4 and IL-10) were noted in patients with CRPS-1, no such profile was observed in our patient. There have been several studies showing clinical improvement after TNF- α antibody administration, either systemically³⁶ or regionally in patients with CRPS.³⁷ There are no recommendations on the use of anti-TNF treatment in patients with CRPS. However, similar to the anti-TNF treatment in patients with Crohn's disease, a loss of response to the treatment may benefit from switching to a different anti-TNF agent or increasing the dose.³⁸

One limitation of our report is that we did not determine the infliximab serum concentrations. Infliximab concentrations are considered to be predictive of a therapeutic response,³⁸ and the serum infliximab level immediately after an infusion is of value in optimizing treatment.⁴⁰ Not all patients with CRPS respond to infliximab treatment. Among the factors implicated in the

lack of response to anti-TNF α treatment are the production of antidrug antibodies that accelerate the drug's clearance, gender, body size, disease type, serum albumin concentration, and the degree of systemic inflammation.⁴¹ The detection of TNF α increases in the serum after treatment with anti-TNF α , as seen in our patient, might be explained by the prolongation of the half-life of infliximab bound to cytokines.⁴² Infliximab, the active ingredient in Remicade[®], is a chimeric IgG1 monoclonal antibody (mAb) with a molecular weight of 149.1 kDa. Free TNF α with a molecular weight of 25.6 kDa is filtered through the glomeruli and cleared into the urine. A complex between TNF α and anti-TNF α has a molecular weight of more than 150 kDa preventing it from being filtered through the glomeruli.⁴³ Reports of increased soluble TNF α have appeared^{44–46} related to paradoxical adverse effects of anti-TNF α therapy. Immune-mediated diseases such as Crohn's disease,⁴⁴ acute anterior uveitis, psoriasis,⁴⁷ lupus-like syndromes,⁴⁸ and cutaneous vasculitis⁴⁹ have been described in patients who have taken anti-TNF α . Thus, these data strongly argue against the utility of monitoring the serum TNF α in guiding therapy with anti-TNF α . Biomarkers of oxidative stress may provide information of the presence of CRPS-1 and may be useful in assessing the efficiency of treatment.

Cytokines and oxidative stress have been implicated in the development of complex regional pain syndrome.^{21–23,26,35,50} In contrast to available data for the anti-TNF α management of rheumatoid arthritis or Crohn's disease, similar data are sparse in patients with CRPS. However, there is evidence that administration of anti-TNF α may provide complete relief of CRPS-1.³⁶ Several studies support the oxidative stress hypothesis speculating that free radical production by mitochondrial dysfunction contributes to the pathophysiology of CRPS-1.^{27,28} Our patient who has CRPS-1 experienced a decrease in allodynia and edema in her affected arms after a single dose of infliximab. Increasing levels of circulating TNF α and a temporary mitigation of oxidative stress as measured by plasma F₂-isoprostane in plasma were observed after the treatment with TNF α antibodies. We were able to demonstrate that 8-iso-prostaglandin F₂ α (8-iso-PGF₂ α), a major F₂-isoprostane (a reliable indicator of in vivo oxidative injury), may be a useful tool in assessing the effectiveness of therapeutic intervention with anti-TNF α in CRPS 1. CRPS is a multifaceted syndrome with several mechanisms leading to a symptom complex, and therapies targeting a single mechanism may be helpful but are rarely totally effective.

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1. [Replace \(Ins\)](#) Tool – for replacing text.

Strikes a line through text and opens up a text box where replacement text can be entered.

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- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
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Strikes a red line through text that is to be deleted.

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- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.

3. [Add note to text](#) Tool – for highlighting a section to be changed to bold or italic.

Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
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Inserts an icon linking to the attached file in the appropriate place in the text.

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- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.

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- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

of the business cycle, starting with the
on perfect competition, constant returns
production. In this environment goods
extra profits and the structure of markets
he model. The New-Keynesian model is
etermined by the model. The New-Keynesian
otaki (1987), has introduced product
general equilibrium models with nominal
ad and supply shocks. Most of this literature

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