Complex Regional Pain Syndrome

An inflammatory disease

Maaike Dirckx

Complex Regional Pain Syndrome: An inflammatory disease

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Voor mijn ouders

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Chapter 1

General introduction

Complex Regional Pain Syndrome (CRPS) is characterized by a continuing regional pain that is disproportionate in time or degree to the usual course of any known trauma or lesion. The pain is regional, not in a specific nerve territory or dermatome, and usually has a distal predominance. CRPS is characterized by a variable progression over time (IASP Committee for Classification of Chronic Pain 2012 (http://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698)).

The first scientific paper on CRPS was published more than 100 years ago by Sudeck[1] and his name was linked to the syndrome for many years (Sudeck's dystrophy). The syndrome has also been referred to by many other terms, but was most commonly called 'reflex sympathetic dystrophy' (RSD). Then, based on a consensus meeting of the IASP in 1993, the term 'complex regional pain syndrome' was agreed upon.[2] CRPS was further divided into type 1 and type 2, with CRPS 1 corresponding to the general image of RSD and CRPS 2 to causalgia.

CRPS is generally characterized by a combination of continuing pain, sensory, vasomotor, sudomotor and motortrophic symptoms. In addition, spontaneous and/or evoked pain and sensory disturbances, such as allodynia and hyperesthesia, changes in skin color and skin temperature, edema, hyper/hypohidrosis, and limited active range of motion are often present. Furthermore, tremor, involuntary movement, muscle spasm, skin, muscle and bone atrophy, and changes in hair and nail growth are reported by patients with this syndrome.[3] For the clinical diagnosis of CRPS, it is currently recommended to use the Budapest criteria.[4] In 2012, these latter criteria were accepted and codified by the IASP Committee for Classification of Chronic Pain (http://www.iasp-pain.org/Education/Content.aspx?ItemNumber = 1698).

The estimated overall incidence rate of CRPS varies from 5.46 to 26.2 per 100,000 person years.[5,6] Females are affected at least three times more often than males. The highest incidence occurs in females in the age group of 61-70 years. The upper extremity is affected more frequently than the lower extremity, and a fracture is the most common precipitating event.[6] Severe CRPS outcome is relatively rare, but incomplete resolution of all signs and symptoms is common and (based on self-reports) only about one-third of the patients reach full recovery.[7] CRPS outcome is worse in patients with the involvement of the upper extremity, a precipitating injury other than a fracture, and in case of 'cold' CRPS.

Although the pathophysiology of CRPS is complex and not completely understood, different underlying mechanisms seem to contribute to the pathophysiology. Referring to its earlier name (RSD), sympathetic dysfunction is one such mechanism.[8,9] Pathology of the sensory somatic nervous system is demonstrated by both peripheral mechanisms, e.g. minimal distal nerve injury affecting nociceptive small fibers[10], and central mechanisms, such as cortical reorganization.[11] Hypoxia has been shown in CRPS[12] and might be caused by endothelial dysfunction.[13] Furthermore, inflammation seems to be

an important mechanism. Potential connections between these separate mechanisms have also been described.[14]

There is considerable evidence for the involvement of inflammation in CRPS; moreover, inflammation seems to play a pivotal role in the pathophysiology of CRPS. CRPS often displays the classic aspects of inflammation, e.g. pain, redness, swelling, warmth and functio laesa.[3] Inflammation can arise from two sources. Classic inflammatory mechanisms can contribute through actions of immune cells (such as lymphocytes and mast cells), which secrete pro-inflammatory cytokines after tissue trauma. Mast cells are known to be involved in CRPS.[15] Neurogenic inflammation may also occur, mediated by release of neuropeptides like calcitonin gene-related peptide[16] and substance-P.[17]

However, local rather than systemic inflammatory responses appear to be relevant in CRPS. Based on the clinical signs and symptoms Sudeck originally proposed the idea of inflammation. In 1993, in a scintigraphic study on CRPS, Oyen et al. demonstrated vascular permeability for macromolecules, a classical phenomenon of inflammation.[18] Tumor necrosis factor alpha (TNF-α) is a cytokine that promotes an inflammatory response. Increased levels of this pro-inflammatory cytokine have been detected in fluid from artificially raised skin blisters in the involved extremity in comparison to the contralateral site.[13,19-21] Also in skin punch biopsies, TNF-α was significantly elevated in CRPS compared to patients with osteoarthritis.[22] A case series showed that TNF-α was only localized in the affected hand of patients with early CRPS using a technetium 99 m-anti-TNF-α antibody scintigraphy.[23] Finally, venous blood of CRPS patients shows normal systemic inflammatory parameters (i.e. normal white blood cell count and C-reactive protein).[24]

In addition to this convincing role for inflammation, several arguments exist for involvement of the immune system in the pathophysiology of CRPS. CRPS shows a beneficial response to treatment in open-label studies on treatment with immunomodulating medication, such as infliximab[25] and immunoglobulin.[26] Furthermore, similar to many other inflammatory diseases, CRPS displays a female predominance[6] and associations with distinct HLA alleles.[27,28]

The aim of the work presented in this thesis was to further explore the immunological aspects of CRPS in order to gain more insight into the pathophysiology of CRPS and the appropriateness of various pharmacotherapeutic interventions.

Pathophysiology

Generally, it is assumed that inflammation is absent in cold CRPS.[14] However, some patients with cold CRPS have displayed full-blown symptoms of warm CRPS after vasodilatation therapy.[29] Therefore, it seems that inflammation could be 'hidden' behind vasomotor disturbances. A study was designed to test this hypothesis.

Autoimmunity is suggested as one of the pathophysiological mechanisms to underlie CRPS. A study was designed to further explore CRPS as a potential autoantibody-associated autoimmune process.

Pharmacotherapeutic interventions

In clinical practice, patients with CRPS are often grouped based on their signs and symptoms, and medication is generally administered depending on these signs and symptoms. Woolf and Decosterd advocated a form of pain treatment based on the mechanisms involved in the pathogenesis of pain.[30] In each patient, the aim should be to identify which mechanisms are responsible for the pain and treatment is then specifically targeted to those mechanisms. Based on this recommendation, it appears that the current strategy applied for therapy may not be correct.

Immunomodulating medication reduces the manifestation of inflammation by influencing the mediators of inflammation, such as cytokines and neuropeptides. Assuming that inflammation plays an important role, it seems more appropriate to correct the baseline inflammatory status to lower disease activity by giving immunomodulating medication. The current empirical evidence for the benefit of administering the most commonly used immunomodulating medication in CRPS was investigated in a systematic review. In addition, a double-blind randomized controlled trial was conducted with the aim to confirm this statement by administering the tumor necrosis factor-α antagonist (i.e. infliximab).

It is unknown whether (apart from the immunomodulating medication) other drugs might also counteract the inflammation. Based on empirical findings on the role of mast cells in the pathophysiology of inflammation in CRPS[15], we investigated the rationale for the use of medication targeting mast cell activity.

The results of the studies presented in this thesis may, hopefully, improve the treatment and outcome of patients suffering from CRPS.

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Chapter 2

Inflammation in cold complex regional pain syndrome

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ABSTRACT

Background

In patients with Complex Regional Pain Syndrome (CRPS) the temperature of the affected side often differs from that of the contralateral side. In the acute phase, the affected side is usually warmer than the contralateral side, the so-called 'warm' CRPS. This thermal asymmetry can develop into a colder affected side, the so-called 'cold' CRPS. In contrast to cold CRPS, in warm CRPS inflammation is generally assumed to be present. However, there are reports of cold CRPS patients, successfully treated with vasodilatation therapy, who subsequently displayed warm CRPS. It seems that inflammation could be 'hidden' behind vasomotor disturbance. This study was designed to test this hypothesis.

Methods

A retrospective analysis was made of patients in our CRPS database. We defined three types of CRPS: cold CRPS, neither cold nor warm (intermediate) CRPS and warm CRPS. Of these patients the difference between the level of the pro-inflammatory cytokines IL-6 (Δ IL-6) and TNF-α (Δ TNF-α) in the affected extremity and that in the contralateral extremity was determined.

Results

The bilateral difference of the level of these cytokines did not differ between patients with cold CRPS, intermediate CRPS or those with warm CRPS

Conclusion

Inflammation may be involved in cold CRPS.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a collection of locally appearing painful conditions following a trauma; these symptoms mainly occur distally and exceed in intensity and duration the expected clinical course of the original trauma, often resulting in considerable restricted motor function.

Especially in the acute phase, CRPS often displays the classic signs of inflammation[1] and there is evidence that local inflammation plays an important role in the pathophysiology of CRPS.[2-5] According to Bruehl, this particularly applies to the acute 'warm' phase.[6]

A transition from a warm/red to a cold/ bluish CRPS presentation is common as CRPS moves from the acute to a chronic stage[6]; however, some patients are cold from the onset.[1]

Although several mechanisms are reported to be responsible for cold CRPS, the mechanism behind the sympathetic dysfunction in CRPS remains controversial.[5] Increased sympathetic outflow (hyperactivity) is one possibility[7] and abnormal sensitivity of adrenergic receptors for normal sympathetic outflow is another.[8]

Nociceptive afferent input may be caused by an increase in the number of alpha 1 adrenoceptors in the affected extremity, increased peripheral alpha adrenergic receptor hypersensitivity, and chemical coupling between the sympathetic and nociceptive neurons in the skin of CRPS-affected limbs.[9] Also, there might be impaired endothelium-dependent vasodilation, indicating endothelial dysfunction.[10] In addition, the nitric oxide/endothelin-1 (NO/ET-1) ratio may be disturbed in the intermediate stage of CRPS, resulting in vasoconstriction.[11] Whether or not vasoconstriction in CRPS is related to the above mechanisms, vasoconstriction might trigger a vicious circle of tissue hypoxia, acidosis and increased production of free radicals.

In cold CRPS, inflammation is generally assumed not to be present.[5] However, there are reports of 'cold' CRPS patients, who displayed full-blown symptoms of 'warm' CRPS after been successfully treated with vasodilatation therapy.[12] It seems that inflammation can be 'hidden' behind vasomotor disturbance.

In pathological situations (like inflammation), circulating cytokines [e.g. tumor necrosis factor (TNF)-α] induce the expression of inducible NO synthase (iNOS) and ET-1 in smooth muscle cells; they also downregulate endothelial NO synthase (eNOS) expression in endothelial cells. Increased NO generated by iNOS can react with superoxide anion to produce peroxynitrite, which can cause further endothelial dysfunction.[13] This imbalance between the NO/ET-1 system is described in cold CRPS.[11]

These findings led to our hypothesis that, (a subgroup of) patients with cold CRPS might still suffer from inflammation. To test this hypothesis, the difference between the level of the pro-inflammatory cytokines IL-6 (Δ IL-6) and TNF-α (Δ TNF-α) in the affected extremity and that in the contralateral extremity was determined. The bilateral differ-

ences were compared between patients with cold CRPS, with neither warm nor cold CRPS and those with warm CRPS.

METHODS

A retrospective analysis was made of data from our CRPS database.

Patients

Sixty-six patients with CRPS in one extremity met the diagnostic criteria of Bruehl et al.[14] All participated in several studies performed between 2001 and 2005. The aim of those studies was to investigate the pathophysiology of CRPS and/or the effects of specific treatments for CRPS.[4] The protocol was approved by the Medical Ethics Committee of the Erasmus MC, P.O. 2040 3000 CA Rotterdam (protocol no. 2001/024) on 1 February 2001.

Measurements

Besides demographic and clinical variables (symptoms and signs), temperature and cytokine levels in artificial skin blisters were determined in both the involved and contralateral extremity.

Temperature measurement

Skin temperature was measured using an infrared tympanic probe thermometer. Measurements were obtained on the dorsal aspect of the hand or foot in a matrix of five points; the difference in mean temperature between the involved and contralateral extremity was calculated.[4]

The measurements were performed at a constant room temperature of 23±0.5°C and the patients had to acclimatize for at least 15 minutes. Psychological state can affect limb temperature. However, the influence of psychological parameters is systemic and therefore will not confound our parameter (bilateral difference in temperature).

We defined three types of CRPS: cold CRPS as an asymmetry cut-off between the affected and contralateral extremity of ≥ -0.6°C, as recommended by Bruehl et al.[15] Intermediate CRPS was defined as a thermal asymmetry between -0.59°C and $< +0.6$°C and warm CRPS as an asymmetry of $\geq +0.6$°C.

Cytokine assays

Levels of the pro-inflammatory cytokines TNF-α and IL-6 were determined in fluid from artificially-derived blisters. Blisters were induced using a suction method. A skin suction chamber was positioned on the skin of the involved and contralateral extremities. A

vacuum of 300 mmHg was applied with an Atmoforte 350A aspirator pump (ATMOS Medizintechnik, Lenskirch, Germany). After 15 min, the vacuum was reduced to 250 mmHg and, after another 15 min, was reduced to 200 mmHg. This negative pressure was maintained for 2-2.5 h. The blisters created were punctured, and fluid was pooled from each side into a 1.5-ml Eppendorf conical polypropylene tube (Eppendorf AG, Hamburg, Germany) and centrifuged for 5 min at 1600 x g.[4]

Blister fluid samples were diluted in appropriate calibrator diluent assay buffer for the direct measurement of cytokines. Cytokine assays were performed following the manufacturer's protocol [PeliKine human ELISA kits for IL-6 (M1906) and TNF-α (M1920); CLB, Amsterdam, The Netherlands]. The standard curve ranges and mean calculated zero signal ± 3 [standard deviation (SD)] were 0-80 and 0.3 pg/ml for IL-6 and 0-1000 and 1 pg/ml for TNF-α, respectively. The absorbance per well was measured at 450 nm with a Medgenix EASIA reader (BioSource Europe S.A., Nivelles, Belgium). Sample concentrations were calculated using the appropriate standard calibration lines and the Softmax® software (Molecular Devices, Sunnyvale, CA, USA) of the reader.[4]

Because the specific distribution of TNF-α and IL-6 in the general population in suction blister fluid is unknown, no data on aberrant levels are available. Therefore, for the present study, the difference between the affected and the contralateral extremity was measured (Δ TNF-α and Δ IL-6). The start of inducing the artificially-derived blisters was always at the end of the morning, so the sample time could not affect any possible differences in cytokine concentrations during the day.

Statistical analysis

Descriptive statistics were used to determine the frequencies of the demographic, clinical and outcome parameters and to describe measures of central tendency and of dispersion, dependent on the shape of their distribution. The Kolmogorov-Smirnov test was used to estimate whether or not the parameters were normally distributed. The difference in IL-6 and TNF-α level between the affected and contralateral side is depicted in a scatter plot.

To estimate whether the parameters differed statistically significant between the three types of CRPS the Kruskal-Wallis (non-normally distributed), One-way analysis of variance (normally distributed) and the Pearson Chi-Square (proportional differences) tests were used.

Analyses were performed using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Of the 66 included patients, data on pro-inflammatory cytokines were available for 48 of them. Based on the temperature asymmetry and the above mentioned cut-off scores, 19 (39.6%) of these patients suffered from a cold CRPS, 20 (41.7%) from an intermediate and 9 (18.7%) from a warm CRPS. Thirty two (66.7%) of the 48 patients were female. Mean age was 46.7 (SD 11.15) years; median duration of CRPS was 5.5 [interquartile range (IQR) 9] months, and median difference in temperature between the affected and contralateral extremity was −2.5°C (IQR 1.33, Range 9). Only gender did not differ between the types CRPS (see Table 1). The clinical phenotypes in terms of symptoms and signs are depicted in Table 2. Of seven patients data were missing. Only the signs of allodynia and hyperesthesia differed significantly between the types of CRPS.

Table 1. Patients' characteristics by type of CRPS

	Type of CRPS			p
	Cold	Intermediate	Warm	
Female gender n (%)	11 (34.4)	14 (43.8)	7 (21.8)	.53
Age / years mean (sd)	42.1 (11.58)	48.1 (10.5)	53.2 (8.07)	.034
Duration / months median (IQR)	12 (27)	4 (7.5)	2 (3.75)	.005

Table 2. Symptoms and signs by type of CRPS

	Symptoms				Signs			
	Cold	Intermediate	Warm	p	Cold	Intermediate	Warm	p
Sensory	n (%)	n (%)	n (%)		n (%)	n (%)	n (%)	
Allodynia	10 (66.7)	11 (64.7)	6 (66.7)	.99	6 (40.0)	13 (76.5)	3 (33.3)	.046
Hyperesthesia	15 (100)	14 (82.4)	8 (88.9)	.24	8 (53.3)	1 (5.9)	4 (44.4)	.01
Vasomotor								
Asymmetry in temperature	15 (100)	17 (100)	9 (100)	1	15 (100)	12 (70.6)	7 (77.8)	.08
Asymmetry in color	15 (100)	17 (100)	9 (100)	1	10 (66.7)	15 (88.2)	8 (88.9)	.24
Sudomotor								
Edema	14 (93.3)	17 (100)	9 (100)	.41	13 (86.7)	13 (76.5)	9 (100)	.27
Asymmetry in sweating	9 (60.0)	14 (82.4)	5 (55.6)	.26	9 (60.0)	5 (29.4)	3 (33.3)	.18
Motortrophic								
Decreased range of motion	15 (100)	15 (88.2)	8 (88.9)	.39	14 (93.3)	15 (88.2)	9 (100)	.55
Weakness	11 (73.3)	13 (76.5)	7 (77.8)	.97	4 (26.7)	4 (23.5)	5 (55.6)	.22
Dystonia	10 (66.7)	9 (52.9)	4 (44.4)	.54	4 (26.7)	5 (29.4)	1 (11.1)	.57
Change hair/ nail growth	10 (66.7)	10 (58.8)	3 (33.3)	.27	7 (46.7)	6 (35.3)	2 (22.2)	.48

The use of anti-inflammatory medication [dimethylsulfoxide, N-acetylcysteine, vitamin C and nonsteroidal anti-inflammatory drug] at the time of inducing the blisters was also

registered. Twelve (63%) of the 19 patients with cold CRPS, 15 (75%) of the patients with intermediate CRPS, and 5 (56%) with warm CRPS used one or more of these medication. These proportions did not differ statistically significant between the types of CRPS. The proportions of the patients using a specific medication also did not differ significantly between the types of CRPS (see Table 3).

Table 3. Medication use at the time of inducing of the blisters

Type of medication	Type of CRPS			p
	Cold (n=19) n (%)	Intermediate (n=20) n (%)	Warm (n=9) n (%)	
Dimethylsulfoxide	9 (47.3)	12 (60)	5 (55.6)	.73
N-acetylcysteine	3 (15.7)	3 (15)	1(11.1)	.95
Vitamin C	0 (0)	1 (5)	0 (0)	.49
Nonsteroidal Anti-Inflammatory Drugs	7 (36.8)	5 (25)	1 (11.1)	.35
Immunomodulating medication	0 (0)	0 (0)	0 (0)	-
No medication	7 (36.8)	5 (25)	4 (44.4)	.54

Note: because some patients used more than one type of medication the percentage of cases does not add up to 100.

The distributions of Δ TNF-α and of Δ IL-6 did not statistically significant differ between the patients with a cold, an intermediate or a warm CRPS (see Table 4 and Figure 1).

Table 4. Median and dispersion of Δ TNF-α and Δ IL-6 by type of CRPS

	Δ TNF-α				Δ IL-6			
	Type of CRPS			p	Type of CRPS			p
	Cold	Intermediate	Warm		Cold	Intermediate	Warm	
Median (IQR)	8.1 (102.4)	12.3 (28.0)	12.3 (28.0)	.99	11.1 (68.0)	44.0 (112.8)	1.6 (63.3)	.16

DISCUSSION

The results of this study show that the three patients groups differing in asymmetrical level of temperature do not significantly differ in asymmetric levels of the pro-inflammatory cytokines TNF-α and IL-6. Likewise, post-hoc pairwise comparison between these groups did not yield a statistically significant difference between these cytokines either, nor comparison of cold versus warm and intermediate as one group (TNF-α, $p = .87$; IL-6, $p = .23$).

Assuming that the patients with a warm CRPS suffer from inflammation and the asymmetric level of the cytokines reflect the presence of inflammation, then our finding

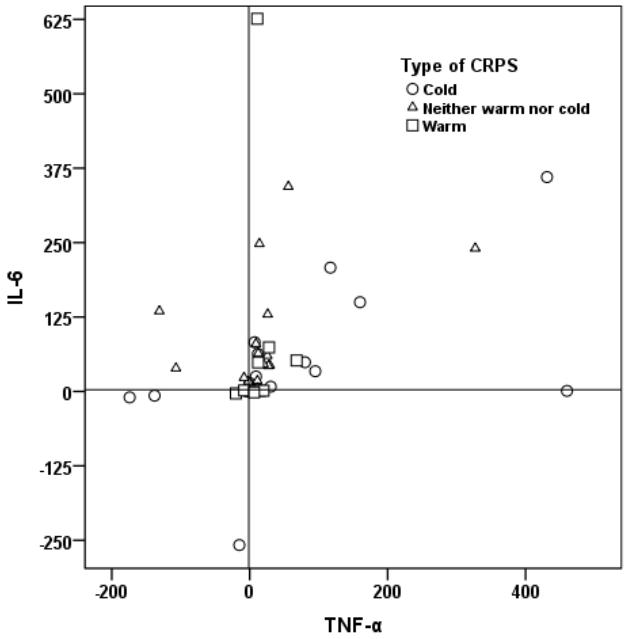

Figure 1. Scatterplot of the difference of IL-6 and TNF-α between the affected and the contralateral side by type of CRPS

is in accordance with the idea that a (subgroup of) patients with cold CRPS might (still) suffer from inflammation.

In clinical practice, patients with CRPS are often grouped (cold vs. warm) based on their signs/symptoms, and medication is generally administered dependent on these signs and symptoms. Our findings suggest that this may be one of the reasons why clinical trials have failed to support the efficacy of many commonly used interventions. Because our findings indicate that the treatment should be based on the underlying mechanism, i.e., irrespective of the thermal asymmetry, inflammation might still be part of the pathophysiology of cold CRPS. Therefore, patients with a cold CRPS and underlying inflammation may benefit more from medication that influences the ongoing inflammation rather than merely vasodilatation therapies.[16]

It is currently not possible to establish the extent to which the individual mechanisms participate in the development of cold CRPS. However, it is possible to determine whether or not there is an ongoing inflammatory process. As suggested, a selection of 2 or 3 representatives from the inflammatory cytokine panel, the Th1/Th2 cytokine panel and the chemokine panel would be sufficient to indicate the inflammatory activity of the CRPS disease.[17] However, obtaining fluid from artificial skin blisters to determine cytokine levels is a time-consuming procedure that limits its routine use for diagnostic purposes in the outpatient clinic.

From a practical point of view we recommend that, irrespective of the amount or direction of existing thermal asymmetry, the concept of (ongoing) inflammation in CRPS should be taken into account when considering therapy. In patients with cold CRPS, if vasodilatation treatment results in an exacerbation of inflammatory signs and symptoms then anti-inflammatory therapy should certainly be considered.

The stability of an increase in asymmetric levels of cytokines is unfortunately unknown. If the stability is low then our measurements could be biased and conclusions should be interpreted accordingly. Other points for discussion are the facts that the duration and age differed significantly between the types of CRPS. The difference in the duration is in accordance with the commonly encountered transition from a warm/red to a cold/bluish CRPS presentation as CRPS moves from the acute to a chronic stage. Although we see no plausible reason for a confounding effect of this difference between the types of CRPS, theoretically it cannot be excluded. The same applies to the difference in age between the types of CRPS.

Patients were treated according to the Dutch guidelines for CRPS.[18] For nociceptive pain treatment, the WHO analgesic ladder is advised and for inflammatory symptoms free-radical scavengers. Immunomodulating medications are not advised. (Some of) the prescribed medication might influence the absolute cytokines levels, it however is unlikely that it might influence the bilateral differences in cytokine levels. Moreover, the proportion of patients using this medication(s) did not differ between the types of CRPS. We therefore are confident that medication use was not a confounder affecting the external validity of our results.

We recommend further research into the pathophysiological mechanism of inflammation in CRPS. If the levels of pro-inflammatory cytokines are measured, this should be done repeatedly.

In conclusion, inflammation might (still) play a role in (a subgroup of) patients with cold CRPS.

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Chapter 3

The prevalence of autoantibodies in complex regional pain syndrome type I

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ABSTRACT

Autoimmunity has been suggested as one of the pathophysiologic mechanisms that may underlie complex regional pain syndrome (CRPS).

Screening for antinuclear antibodies (ANA) is one of the diagnostic tests, which is usually performed if a person is suspected to have a systemic autoimmune disease. Anti-neuronal antibodies are auto-antibodies directed against antigens in the central and/or peripheral nervous system. The aim of this study was to compare the prevalence of these antibodies in CRPS patients with the normal values of those antibodies in the healthy population.

Twenty seven (33%) of the 82 CRPS patients of whom serum was available, showed a positive ANA test. This prevalence is significantly higher than in the general population.

Six patients (7.3%) showed a positive result for typical anti-neuronal antibodies. This proportion, however, does not deviate from that in the general population.

Our findings suggest that auto-antibodies may be associated with the pathophysiology of CRPS, at least in a subset of patients. Further research is needed into defining this subset and into the role of auto-antibodies in the pathogenesis of CRPS.

INTRODUCTION

Complex Regional Pain Syndrome (CRPS) is a collection of locally appearing painful conditions following trauma which chiefly occur distally and exceed in intensity and duration the expected clinical course of the original trauma.

The pathophysiology is complex and still not completely understood. It is reasonable to assume that different mechanisms, e.g. inflammation, hypoxia, central sensitisation and neuroplasticity, are involved in a complex network of interactions, resulting in a broad range of signs and symptoms.[1]

The involvement of the immune system in the pathophysiology of CRPS is appreciated for several reasons. First, CRPS shows several clinical characteristics of an inflammatory disease, including pain, redness, swelling and warmth.[2] Additionally, levels of pro-inflammatory cytokines are elevated in blister fluid from CRPS affected limbs.[3,4] CRPS shows a beneficial response to treatment with inhibitors of inflammation, such as corticosteroids.[5]

Complementary is the fact that, similar to many other chronic inflammatory diseases, CRPS displays a female predominance[6] and associations with distinct HLA alleles.[7-9] The incidence of CRPS is higher in patients with chronic inflammatory disorders, such as asthma[10] and multiple sclerosis.[11]

Autoimmunity has been suggested as one of the underlying mechanisms in the pathophysiology of CRPS. There are several arguments that point in this direction. First, IgA-antibodies to Campylobacter were present in CRPS patients with short disease duration[12] and an increased seroprevalence of Parvovirus B19 in CRPS patients compared to controls has been reported.[13,14] Both infectious agents have previously been implicated in the induction of autoimmune diseases.

Second, immunohistochemistry has revealed the presence of auto-antibodies against nervous system structures in at least a part of the CRPS-patients, included in a study by Blaes et al.[15] Another study showed that about 30-40% of CRPS patients have surface-binding auto-antibodies against an inducible autonomic nervous system auto-antigen.[16] Third, a subgroup of CRPS patients, i.e. those who developed CRPS with only a minimal preceding trauma, showed a much stronger immune response against nervous tissue compared to the whole group.[12] Fourth, animal studies have demonstrated that the transfer of IgG antibodies from CRPS patients to mice causes abnormal behaviour and motor function in these mice.[17] And finally, treatment with intravenous immunoglobulin can reduce pain in refractory CRPS.[18]

These results suggest that CRPS is associated with autoimmunity, including an auto-antibody-mediated immune process, at least in a part of the patients. Interestingly, CRPS is even considered as prototype of a novel kind of autoimmune disease.[19]

Autoimmune diseases are often associated with an increased prevalence of positive testing for antinuclear antibodies (ANA). These auto-antibodies are reactive with anti-

gens in the nucleoplasm. ANA are probably present in the circulation of all human beings, but the employed test is considered positive when titres are elevated significantly above the normal serum level.[20] Screening for ANA is one of the diagnostic tests which is usually performed if a person is suspected to have a systemic autoimmune disease.[21]

Anti-neuronal antibodies, often called "onconeural antibodies" given their paraneoplastic nature in many cases, are auto-antibodies directed against antigens in the central and/or peripheral nervous system. Anti-neuronal antibodies against intracellular antigens in general are not thought to be pathogenic. On the contrary, the anti-neuronal antibodies directed against cell surface antigens are themselves disease mediating. In contrast to what the name "onconeural" suggests, anti-neuronal antibodies are not strictly related to cancer.[22]

The aim of the present study was to further explore CRPS as a potential auto-antibody-associated autoimmune process. For this purpose, we compared the prevalence of CRPS patients with a positive test for antinuclear antibodies and for anti-neuronal antibodies with the prevalence in the healthy population.

MATERIALS AND METHODS

This study was approved by the Medical Ethics Committee of the Erasmus MC Rotterdam (MEC-2012-037).

Patients

Our department, a university Center for Pain Medicine serves as an expert center for CRPS patients. Both acute and chronic CRPS patients visit the clinic on their own initiative or on referral by GP's or medical specialists. There is a weekly outpatient clinic especially for CRPS patients, led by physicians with clinical and research experience in CRPS.

All patients who visited the Center for Pain Medicine between 2001 and 2007, and fulfilled the Harden-Bruehl diagnostic criteria for CRPS[23] were invited to participate in on-going CRPS studies.

For all patients signs (subjective) and symptoms (objective), i.e., the presence or absence of allodynia, hyperalgesia, dystonia, bilateral difference in temperature, difference in colour, difference in sweating, difference in motor range, difference in strength, were recorded.

In each patient who participated in a study, venous blood was drawn. Serum of this blood was stored with permission, to use in future research. For the current study, serum of 82 patients was available. All these patients were classified as CRPS type 1. The characteristics of the 82 patients are described in table 1.

Table 1. Patients' characteristics

Characteristics	n=82
Woman (n,%)	69 (84.1)
Age in years (mean, sd)	44.2 (12.37)
CRPS duration in months (median, IQR)	11 (36-5)
Cold CRPS (n, %)	44 (53.7)
Warm CRPS (n, %)	31 (37.8)
Unknown (n, %)	7 (8.5)
Upper limb (n, %)	48 (58.5)
Precipitating injury	
Trauma (n, %)	53 (64.6)
Operation (n, %)	21 (25.6)
Spontaneous (n, %)	6 (7.3)

Laboratory tests

Venous blood samples were centrifuged immediately after collection at 3000 rpm during 10 minutes and serum was stored at minus 80 degrees Celsius until use.

Anti-nuclear antibodies (ANA) were determined according to international recommendations[24] with a commercially available test system, according to manufacturer's instructions. Briefly, HEp-2000™ cells (Immuno Concepts, Sacramento, CA) were incubated with 1:80 diluted patient serum. After washing, bound autoantibody was detected using fluorescein (FITC)-conjugated anti-human IgG (Immuno Concepts) and visualized at 488 nm by fluorescence microscopy. ANA results were considered positive if at least weak positive nuclear staining of HEp-2000™ cells was observed. Borderline results were discarded.

Anti-neuronal antibodies were determined according to international guidelines[25] with a commercially available test system, according to manufacturer's instructions.

Briefly, primate cerebellar cryosections (IMMCO Diagnostics, Buffalo, NY) were incubated with 1:400 diluted patient serum. After washing, bound autoantibody was detected using fluorescein (FITC)-conjugated anti-human IgG (IMMCO Diagnostics) and visualized at 488 nm by fluorescence microscopy. Results were considered positive if at least weak positive staining of neuronal nuclei (anti-neuronal nuclear antibody, ANNA) or Purkinje cells (Purkinje cell cytoplasm antibody, PCA) was observed. Borderline results were discarded as well as false positive neuronal nuclear staining in the presence of a positive ANA.

Since literature reference varies regarding prevalence of auto-antibodies in healthy individuals, mostly due to methodology, we did not use literature reference for comparison. Instead, the results of ANA and anti-neuronal antibody obtained in CRPS patients were compared to those we obtained ourselves in parallel using serum obtained from

randomly selected healthy blood bank donors, using identical methodology as described above.

Statistical analysis

Descriptive statistics were used to determine the (Multiple Response) frequencies of the demographic variables and the outcome parameters and to describe measures of central tendency and of variability, dependent on the shape of their distribution. Testing for normality of the distributions was performed using the Kolmogorov-Smirnov test. The difference between the proportion CRPS patients with positive (nuclear or neuronal) antibodies and that in the healthy population was analysed using the Fisher's Exact Test, 2-sided. The same test was applied to evaluate the difference in proportion of signs and symptoms between (1) the patients positive and those negative for anti-nuclear antibodies and (2) between the patients positive and those negative for anti-neuronal antibodies.

Difference in duration of the CRPS between patients with positive and those with negative anti-nuclear antibodies were tested using the Mann-Whitney U test. Analyses were performed using IBM SPSS Statistics 21.

RESULTS

Twenty seven (33%) of the 82 included CRPS patients showed a positive result for anti-nuclear antibodies. This proportion is significantly higher compared to that in the healthy population (n=90), in which we observed 4% ANA positivity ($P < .001$).

The observed ANA immunofluorescence patterns were diverse, including homogeneous, speckled and nucleolar patterns. See table 2.

Table 2. IF pattern of Anti-Nuclear Antibodies

Anti-Nuclear Antibodies positive	n=27
Homogeneous (n, %)	7 (26)
Speckled (n, %)	6 (22)
Nucleolar (n, %)	12 (44)
Homogeneous and nucleolar (n, %)	2 (8)

No statistically significant difference was found in the proportion of patients with an ANA positivity between patients with a cold and those with a warm CRPS, respectively 34.1% and 32.3% (p=0.99). Likewise, no statistically significant difference in duration of the CRPS was found between the patients with a positive test for ANA and those with a negative test (p=0.66).

Six (7.3%) of the 82 included CRPS patients showed a positive result for anti-neuronal antibodies. This percentage does not deviate from that in the healthy population (7.5%).

As indicated by the immunofluorescence pattern on the cerebellum substrate, the majority of reactivity was directed against neuronal nuclei (ANNA). In addition, reactivity against Purkinje cell cytoplasm (PCA) was observed. See table 3. However, the immunofluorescence pattern in the healthy population indicated reactivity to basket neurons and/or neurofilaments, as opposed to the ANNA and PCA patterns observed in the CRPS patients.

Table 3. IF pattern of Anti-Neuronal Antibodies

Anti-Neuronal Antibodies positive	n=6
Neuronal nuclei (n, %)	4 (66)
Purkinje cells (n, %)	1 (17)
Neuronal nuclei and purkinje cells (n, %)	1 (17)

Two patients showed both a positive ANA test (speckled pattern) and a positive result for anti-neuronal antibody (ANNA).

No statistically significant differences in signs or symptoms between patients positive and those negative for anti-nuclear antibodies were found. The same applied to patients positive and those negative for anti-neuronal antibodies. For all proportional differences $0.13 = p \leq 1$.

DISCUSSION

To gain more insight in the potential role of systemic and/or organ-specific autoimmunity in the pathophysiology of CRPS, we studied the prevalence of both anti-nuclear antibodies (ANA) and anti-neuronal antibodies in CRPS patients.

The reported prevalence of ANA in healthy individuals is up to 20% or more. The prevalence of ANA depends, however, on various factors including age, gender and methodology.[26] Using our method of ANA testing, the prevalence in healthy individuals was 4%.

In our CRPS study sample we found a statistically significant higher positive ANA prevalence (33%) compared to that in the healthy population. To correct for a possible confounder age, since the prevalence of positive testing for ANA in the general population is higher amongst people aged above 65 years (up to 30%), we excluded the CRPS patients aged above 65 years (two patients). The positive ANA prevalence in CRPS remained significantly higher, 30%.

Diverse ANA patterns were observed in CRPS, including homogeneous, speckled and nucleolar. Either pattern can be observed in systemic autoimmune disease, but is not specific to any particular autoimmune disease.[27]

The prevalence of anti-neuronal antibodies in CRPS patients was 7.3%, showing characteristic ANNA and PCA patterns.[25,28] A similar prevalence was found in healthy subjects, however showing a clearly different, atypical pattern. The clinical relevance of such patterns is unclear, but are observed more often in subjects without apparent neurological disease (Schreurs MWJ, unpublished results). Although the immunofluorescence pattern in the healthy population, reactivity to basket neurons and/or neurofilaments, was different as compared to the ANNA and PCA patterns observed in CRPS patients, this observation may lack meaning due to the low amount of positive patients identified.

The phenotype does not seem to be different, because the signs and symptoms did not show any significant differences between CRPS patients positive or negative for anti-nuclear antibodies, nor for anti-neuronal antibodies.

Our findings suggest that auto-antibodies may be associated with the pathophysiology of CRPS, at least in a part of the patients. However, although increased compared to the general population, the positive ANA prevalence in CRPS patients is much lower than in patients with classic systemic autoimmune disease such as systemic lupus erythematosus (SLE), that shows a prevalence of 99%.[21] The positive ANA prevalence in CRPS patients is more in line with the 25% observed in patients with autoimmune diseases such as rheumatoid arthritis (RA).[21] Based on these findings, we may suggest that CRPS is more similar to an autoimmune disease as RA than to a systemic autoimmune disease as SLE. This hypothesis is supported by studies that revealed associations between CRPS and chronic inflammatory disorders, including asthma[10] and multiple sclerosis.[11] To our knowledge there are no reports of strong associations between CRPS and autoimmune diseases, although there are two case-reports of co-occurrence of the two disorders in one patient.[29,30]

The presence of anti-neuronal antibodies in CRPS patients has been established in earlier research.[15,16] In previous studies, antibodies were directed against a neuroblastoma cell line. In our current study we used a cerebellum substrate containing both afferent and efferent nerve pathways, with sensory and motor function. We chose this substrate because it resembles peripheral nerve tissue, which seems to be affected in CRPS.[31] Therefore, and based on our observation of characteristic ANNA and PCA reactivity in some patients, our results suggest that autoimmunity against the peripheral nerve system could be of relevance in CRPS in a limited subset of cases. Since the majority of CRPS patients did not display anti-neuronal antibodies, a causal relationship remains to be determined. Alternatively, in the subset of patients with anti-neuronal antibodies, their expression might have been a secondary event as a result of nerve damage.[32] It

would therefore be interesting to search for signs of actual nerve damage in patients who display these anti-neuronal antibodies and to search for the actual antigenic specificity of the antibodies. To define whether or not anti-neuronal antibodies could be causative for CRPS is of clinical relevance, as immune therapies, such as corticosteroids and intravenous immunoglobulin have been shown to positively affect the neurological outcome when a disorder is caused by an anti-neuronal antibody directed against a cell surface antigen.[22] Interestingly, previous work has already shown that some CRPS patients do respond to intravenous immunoglobulin treatment.[18]

Before speaking of clear evidence of an autoimmune etiology, Witebsky's criteria for an autoimmune disease should be considered.[33] These criteria include: 1) demonstration of a specific antigen; 2) circumstantial evidence of an autoimmune or inflammatory disorder from clinical clues; and 3) reproduction of clinical features in recipient animals by passive transfer of putatively pathogenic antibodies. We argue that CRPS definitely meets the second criterion. There are indications that the first criterion is met as well, however this applies only to some of the CRPS patients. And more research is needed to define specific antigens involved. Injection of serum-IgG from a CRPS patient into groups of mice showed abnormal physical behavior and a significant reduction in rearing.[34] However, these findings are not a reproduction of the clinical features, as needed for the third criterion. Therefore, although suggestive, it remains uncertain whether CRPS can be defined as an autoimmune disease.

In conclusion, our findings indicate an autoimmune-related pathophysiology of CRPS in at least a subgroup of CRPS patients. Further research is needed into defining this subset and into the role of antibodies in the pathogenesis of CRPS in these patients.

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Chapter 4

Effect of immunomodulating medications in complex regional pain syndrome

A systemic review

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ABSTRACT

Background

Different mechanisms are involved in a complex network of interactions resulting in the painful and impairing disorder CRPS. There is convincing evidence that inflammation plays a pivotal role in the pathophysiology of CRPS. Immunomodulating medication reduces the manifestation of inflammation by acting on the mediators of inflammation. Therefore, as inflammation is involved in the pathophysiology of CRPS, immunomodulating medication in CRPS patients may prove beneficial.

Objectives

To describe the current empirical evidence for the efficacy of administering the most commonly used immunomodulating medication (i.e. glucocorticoids, TNF-α antagonists, thalidomide, bisphosphonates and immunoglobulins) in CRPS patients.

Methods

PubMed was searched for original articles which investigated CRPS and the use of one of the above-mentioned immunomodulating agents.

Results

The search yielded 39 relevant articles: from these, information on study design, sample size, duration of disease, type and route of medication, primary outcome measures and results was examined.

Discussion

Theoretically, the use of immunomodulating medication could counteract the ongoing inflammation and might be an important step in improving a disabled hand or foot, leading to further recovery. However, more high-quality intervention studies are needed.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a complication after surgery or trauma, but spontaneous development is also described. It was formerly known by many names, but was most commonly referred to as 'reflex sympathetic dystrophy' (RSD).

The diagnosis of CRPS is based on signs and symptoms. Of the several diagnostic criteria sets available, the most used are the Veldman[1], the IASP[2] and the 'Budapest Criteria'.[3]

Most patients have a burning spontaneous pain, disproportionate in intensity to the initial eliciting event, most often being a fracture of an extremity.[4] In the acute stages of CRPS the affected limb is generally warmer than the contralateral limb, with edema as a common symptom. Hypo- or hyperhidrosis is present in many patients. About 70% of the patients have weakness of all muscles in the affected region and a decrease in the active range of motion. The upper extremities are affected more frequently than the lower extremities.[5]

The estimated overall incidence rate of CRPS is 26.2 per 100,000 person years.[5] Females are affected at least three times more often than males. The highest incidence occurs in females in the age category of 61-70 years.[5]

It is reasonable to assume that different mechanisms are involved in a complex network of interactions, resulting in the painful and impairing disorder of CRPS.[6] CRPS often displays the classic aspects of inflammation.[1] There is convincing evidence that inflammation is one of the mechanisms playing a pivotal role in the pathophysiology of CRPS.[6] The presence of local inflammation was shown in a scintigraphic study on CRPS in which vascular permeability for macromolecules was demonstrated.[7] Increased systemic CGRP levels in patients with acute CRPS suggest neurogenic inflammation as a pathophysiologic mechanism.[8] Increased levels of the pro-inflammatory cytokines have been detected in fluid from artificially raised skin blisters in the involved extremity in comparison to the contralateral site; however, no correlation was found between levels of pro-inflammatory cytokines and the characteristics or duration of the disease.[9-12] This is an indication that inflammation explains a part, but not the whole picture of the pathophysiology.

Analysis of blister fluid with a multiplex array (testing for 25 different cytokines) revealed a pro-inflammatory expression profile, with increased markers for activated monocytes and macrophages.[13] Also, a pro-inflammatory cytokine expression profile was demonstrated in the cerebrospinal fluid of CRPS patients.[14] Venous blood of patients with CRPS showed elevated mRNA levels of the pro-inflammatory cytokines TNF and IL-2 and serum IL-2 protein, as well as a reduction of mRNA levels of the anti-inflammatory cytokines IL-4 and IL-10.[15] Plasma demonstrated higher levels of soluble TNF-α receptor.[16] After performing technetium 99m-anti-TNF-α antibody scintigraphy, a recent case

report showed that TNF-α was only localized in the affected hands of patients with early CRPS.[17] In addition, the contribution of inflammation in the pathophysiology of CRPS is suggested by the successful reports from open-label studies on treatment with immunomodulating agents such as infliximab[18] and immunoglobulin.[19]

Immunomodulating medication reduces the manifestation of inflammation by influencing mediators of inflammation, such as cytokines, neuropeptides, eicosanoids and amino acids. If inflammation does play a role in the pathophysiology of CRPS, then immunomodulating medication may be beneficial for CRPS patients.

Despite the fact that, especially in higher doses, nonsteroidal anti-inflammatory drugs (NSAIDs) also show anti-inflammatory effects, these drugs are not included in the group of immunomodulating medications. For this reason, we disregarded them from this review. In general we know that NSAIDs have no effect in the CRPS.[20] In the Netherlands there is some popularity for treating CRPS with free radical scavengers.[21] Due to a lack of convincing evidence for effectiveness, these drugs never gained general international acceptance. For this study we decided to exclude them.

This review presents the current empirical evidence for the benefit of administering the most commonly used immunomodulating drugs in CRPS patients.

Glucocorticoids

Glucocorticoids are anti-inflammatory by preventing phospholipid release, decreasing eosinophil action and a number of other mechanisms. Interactions between the nervous system, the hypothalamic-pituitary-adrenal axis, and components of the innate and adaptive immune system play a key role in the regulation of inflammation and immunity. Glucocorticoids can also inhibit prostaglandin production through some independent mechanisms.[22]

Tumor necrosis factor-α antagonists

Tumor necrosis factor alpha (TNF-alpha) is a cytokine which promotes an inflammatory response. Although principally produced by macrophages, other cells (including lymphocytes and mast cells), and tissue cells (such as epithelial cells and fibroblasts) can also secrete TNF.[23] The possible mechanism of action of anti-TNF agents are inhibition of inflammatory 'cytokine cascade' mediated by TNF; sequestration of TNF by binding; complement-mediated lysis of cells expressing TNF; altered leukocyte recruitment and endothelial activation; reduction of vascular endothelial growth factor expression and neovascularization; restoration of function of regulatory T cells, and induction of T lymphocyte apoptosis.

Thalidomide

Thalidomide inhibits TNF-α production by human blood monocytes, without influencing either general protein synthesis or the expression of three other monocyte-derived cytokines. Thalidomide exerts a selective effect by suppressing only TNF-α secretion, neither IL-1β, IL-6, nor granulocyte macrophage colony-stimulating factor production is influenced by the drug.[24] Thalidomide was introduced as a sedative drug in the late 1950s. It was withdrawn from the market in the early 1960s due to teratogenicity and neuropathy. There is growing interest due to its immunomodulatory properties. Thalidomide is also a potent inhibitor of new blood vessel growth.[25] On the basis of this finding clinical trials were initiated, which have reported its effectiveness against multiple myeloma.[26]

Bisphosphonates

The most important biological effect of bisphosphonates is the reduction of bone remodeling through the inhibition of osteoclastic activity, but there is evidences of extraskeletal biological effects of bisphosphonates.[27] Bisphosphonates exert their effects also on cells of the immune system with an "immunomodulating" effect, influencing the production of pro- and anti-inflammatory cytokines and changing the molecular expression involved in the immune process and anti-inflammatory response. The exact identification of target cells and interference mechanisms of bisphosphonates with the immune and inflammatory responses are not yet totally clear.

Immunoglobulins

The mechanism of action of immunoglobulins involves modulation of expression and function of Fc receptors, interference with activation of complement and the cytokine network, provision of anti-idiotypic antibodies, regulation of cell growth, and effects on the activation, differentiation, and effector functions of dendritic cells, T and B cells.[28] Modulation of the production of cytokines and cytokine antagonists by intravenous immunoglobulin is a major mechanism by which immunoglobulin exerts its anti-inflammatory effects. The anti-inflammatory effects are not restricted to monocytic cytokines, but are also largely dependent on the ability of intravenous immunoglobulin to modulate Th1 and Th2 cytokine production.

MATERIALS AND METHODS

The PubMed database was searched from inception up to end August 2010. The search was for original articles (in the English language) which met our criteria. The initial search strategy included ((complex regional pain syndrome [Title/Abstract] OR reflex

sympathetic dystrophy [Title/Abstract]) AND (glucocorticoids/steroids [Title/Abstract]) OR (TNF-α antagonist/anti-TNF [Title/Abstract]) OR (thalidomide [Title/Abstract]) OR (bisphosphonate/biphosphonate [Title/Abstract]) OR (immunoglobulin [Title/Abstract])).

The abstracts of retrieved articles were manually reviewed to assess suitability for inclusion using the following criteria: adult humans having CRPS (the previously used names for this syndrome were also allowed, e.g. shoulder-hand syndrome, RSD), together with the use of one of the abovementioned immunomodulating medications. The references of the selected articles were also checked for additional relevant papers.

Finally, from all studies fulfilling the inclusion criteria the following information was examined: type of study, sample size, duration of disease, type and route of medication, primary outcome measures, and results.

RESULTS

The literature search yielded 39 articles, 10 case reports, 19 observational studies, and 10 randomized controlled trials (RCTs: 7 blinded and 3 non-blinded).

The results of the various medications are described below (and in Table 1).

Glucocorticoids

A total of 3 case reports, 13 open-label studies and 5 RCTs (2 of which blinded) were found.

The 3 case reports described 5 patients: in all cases the signs and symptoms improved after administration of glucocorticoids.[29-31]

In the 13 open-label studies, various dose regimens were prescribed and different routes of administration were used.[32-44] In 3 of the open-label studies, patients who received medication were analyzed, as were those who received stellate ganglion blockade, physiotherapy, or no specific treatment. These treatments were then compared with each other.[33, 34, 38] Although the results of the open-label studies were based on different parameters, like clinical improvement and visual analog scale, the use of glucocorticoids seems to cause predominantly improvement in outcome. Only one of these studies described 2 major adverse events (arterial occlusion below the femorals and manic psychosis[33]); in the remaining studies only minor events (e.g. weight gain) were described.

Of the 5 RCTs[45-49] 2 were double-blinded.[47,49] The first double-blinded study showed no improvement of CRPS using a Bier block with methylprednisolone compared with placebo.[47] The second study, in which patients received medication intrathecally, was stopped early owing to no effect after interim analysis.[49]

In 2 of the remaining 3 non-blinded RCTs, use of glucocorticoids resulted in a significantly greater improvement in activity of CRPS[45] or in shoulder-hand syndrome score[46] compared with placebo. The third RCT showed a significantly greater improvement in the signs and symptoms of CRPS among patients receiving glucocorticoid compared with those receiving piroxicam.[48]

In 3 of the 5 RCTs, the patients suffered from CRPS for a period of about 3 months.[45, 47, 48] In another study, patients suffered for a mean duration of 4.5 years,[49] and in 1 study the duration of disease was not reported.[46] The studies used different primary outcome measures. In 1 RCT, the placebo group could also receive medication afterwards[46] (Table 1). In contrast to the open-label studies, no serious side-effects were described.

TNF-α antagonists

Two case reports were found describing 3 patients.[18, 50]

All 3 patients received infliximab and showed improvement in pain, temperature and motor function. The 2 patients who had CRPS for 2 to 3 months showed greater improvement than patients with CRPS for 5 years. No adverse effects were observed.

Thalidomide

Two case reports and 1 open-label study were found.

In the case reports, thalidomide was introduced for CRPS patients with a comorbid condition.[51, 52] In this case thalidomide had a beneficial effect on CRPS. In the open-label study 42 patients were treated.[53] A "dramatic response" occurred in 17% of the patients, and 14% experienced at least modest pain relief and/or showed some reduction in the need for concurrent medications. No results for the remainder of the patients were reported.

In 1 patient, due to persistent paresthesia, thalidomide was temporarily stopped after which the pain re-occurred.[52] Although patients often felt worse during the first weeks of therapy (e.g. increased pain and edema) no major side-effects were reported.

Bisphosphonates

Two case reports, 4 open-label studies, and 4 double-blind RCTs were found.

In the case reports the 2 patients experienced pain relief.[54, 55]

In the open-label studies pamidronate or ibandronate was used.[56-59] These studies reported a positive effect of both drugs on pain intensity.

Patients who participated in the RCTs were prescribed alendronate (oral or intravenous)[60,61], clonadrate[62] or pamidronate.[63] All were compared with placebo. In 2 of the RCTs, patients had CRPS for less than 6 months[61, 62], compared with about 7 months to 6 years in the other 2 studies.[60, 63] In all RCTs there was a significant decrease of pain. Apart from pain, the other primary outcome measures were different but all showed improvement. Three RCTs were followed by an open-label study in which continuation

Table 1

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
Glucocorticoids									
1953	Russek	OL	17	6.5 weeks	Cortisone	oral or im	Clinical improvement	5 complete relief of signs and symptoms, 8 marked improvement, 3 moderate improvement and 1 no response	USA
1953	Steinbrocker	OL	13 14		Corticotropin/ cortisone or both vs. sympathetic block		Clinical features (pain, signs, swelling, trophic changes), graded: complete recovery, greatly improved, slightly improved or no improvement	All symptoms and signs were abolished in 4, great improvement in 4, 1 failed to respond. Recovery function depended on stage disease, complete relief of shoulder or hand pain in all but 2 patients.	USA & Canada
1957	Rosen	OL	15 7 20 31	1day-4 years	ACTH/cortisone vs. stellate ganglion block, physiotherapy, other or no specific treatment		Grading of results of treatment: excellent, good, fair or poor	10 of 15: excellent or good result; 1 of 7: excellent or good response; 9 of 20: excellent or good; none: excellent or good	Canada
1973	Glick	OL	17		Prednisolone	oral	Clinical improvement: poor, no improvement, good, very good, excellent	Only three failed to derive any benefit	UK
1974	Mowat	CR	3	2-7 months	Prednisolone Hydrocortisone	local in bursa		Reduction in volume, improvement in all other symptoms & signs relieve of pain	UK

Table 1 (continued)

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
1976	Glick	OL	21		Prednisolone or Methylprednisolone/ ACTH	oral or im	Improvement, grading very good, good, fair and poor	Relief of pain, >50% improvement of function: 10 Constituted reduction of pain, 20% improvement in range of movement: 3 Relief of pain but still requiring analgesics, no improvement of movement: 5 No significant change: 3	UK
1976	Kozin	OL	11	4-60 weeks	Prednisone		Shoulder range of motion, grip strength, tenderness and ring size	In 4 patients: improvement in all measurements, significant for swelling and tenderness.	USA
1981	Kozin	OL	55	75.9 ±67.9 weeks	Prednisone vs stellate ganglion blockade	oral	Subjective estimate: poor, fair, good or excellent	Prednisone: 63% a good to excellent response Stellate blockade: fair 15%, poor 85%	USA
1982	Christensen	RCT	23	3 months	Prednisone vs placebo	oral	Activity of RDS: pain, edema, volar sweating and finger-knitting ability	All prednisone-treated: more than 75% response to treatment Placebo: 2 of 10 had improvement	Denmark
1983	Poplawski	OL	27	2-36 months	Methylprednisolone	ivrb	Grading: excellent, very good, good, fair, poor	21 of 28 extremities improved significantly: 11 excellent, rest substantial improvement; 7 poor results.	Canada
1987	Dirksen	CR	1	3 months	Methylprednisolone	cervical epidural		Marked pain relief, improved motor control, reduced muscular contracture and trophic changes occurred	The Netherlands

Table 1 (continued)

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
1991	Tountas	OL	17	<6 months	Methylprednisolone	ivrb	Grading: excellent, good, fair and poor	Overall late results: excellent: 9, good: 2 and fair: 4 patients.	
1994	Braus	RCT[1]	36		Methylpredisolone vs. placebo	oral	Shoulder-Hand Syndrome Score	Placebo: no significant improvement; 34 treated with corticoids: 31 of them symptom free	Germany
1996	Grundberg	OL	47	8-36 weeks	Methylprednisolone	im	Pain, motion PIP joint, swelling, pinch strength	In all patients: relief of night & rest pain, improvement of motion in PIP joint, swelling improved	
1998	Zyluk	OL	36	1-8 months	Methylprednisolone	ivrb	Overall results, graded good, moderate or poor.	Good: 25 patients; moderate: 8; poor: 3	Poland
2002	Okada	CR	1	>3 months	Methylprednisolone			Symptoms were dramatically improved	Japan
2004	Taskaynatan	RCT	22	3.1 ±1.4 months	Methylprednisolone vs. placebo	ivrb	VAS, range of motion and volumetric edema	No benefit in both groups	Turkey
2006	Kalita	RCT	60	7-100 days	Prednisolone vs. piroxicam	oral	CRPS-score	Prednisolone: improvement 83.3%; Piroxicam: 16.7%	India
2006	Bianchi	OL	31	10-204 days	Prednisone		VAS, clinical severity (scale 0-22)	VAS: reduction of score Clinical severity: significant improvement	Italy
2008	Zyluk	OL	75	< 4 months	Dexamethasone	iv	VAS; Loss of finger flexion, grip strength; CRPS-score	Mean VAS decreased; mean loss of finger flexion decreased, grip strength did not improve; CRPS score decreased	Poland
2010	Munts	RCT	21	4.5 years	Methylprednisolone vs. placebo	intrathecal	Change in pain	No effect on pain--> trial stopped prematurely	The Netherlands

Table 1 (continued)

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
TNF-α antagonists									
2004	Huygen	CR	2	2-months & 5-years	Infliximab	iv	Clinical examination: pain, temperature, edema, motor function	1 slight improvement and 1 considerable improvement	The Netherlands
2007	Bernateck	CR	1	3 months	Infliximab	ivrb	Pain, temperature, hand grip strength, ROM wrist and QST	Substantial improvement of pain intensity, temperature difference and range of motion.	Germany
Thalidomide									
2001	Rajkumar	CR	1	3 years	Thalidomide			Improvement and near resolution of symptoms	USA
2003	Ching	CR	1	6 years	Thalidomide			Pain and other symptoms disappeared	New Zealand
2003	Schwartzman	OL	42	long-standing	Thalidomide		Objective and subjective responses including increased function, healing of lesion, pain reduction and lower analgesic requirements	17% "dramatic responses" 14% modest pain relief and/ or some reduction in need for medication	USA
Bisphosphonates									
1995	Maillefert	OL	11	>6 months	Pamidronate	iv	VAS and Physical global assessment	Mean VAS decreased 4: no improvement/ 1: moderate improvement/ 3: significant improvement/ 3:excellent improvement	France
1997	Cortet	OL	23	15±13 months	Pamidronate	iv	Decrease of pain (VAS and PVS)	Significant decrease of VAS and PVS: day 0 and day 30/ day 0 and day 60/ day 0 and day 90.	France

Table 1 (continued)

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
1997	Adami	RCT[1]	20	5-34 weeks	Alendronate vs. placebo	iv	VAS; arbitrary score of motion and circumference of affected joints	Diminution in VAS, tenderness and swelling; improvement in motion significantly different	Italy
2000	Varenna	RCT[1]	32	4.0 ±2.3 months	Clonadrate vs. placebo	iv	VAS	Significant decrease	Italy
2000	Siminoski	CR	1	> 1 year	Pamidronate	iv		pain decrease--> gone	Canada
2001	Kubalek	OL	29	41.89 ±38.90 weeks	pamidronate	iv	Complete disappearance of pain Functional improvement: increase in range of movement more than 20	25 patients (86,2%) 14 patients (70%)	France
2004	Robinson	RCT	27	3 months-6 years	Pamidronate vs. placebo	iv	VAS; patient's global assessment of disease severity; functional assessment	VAS: overall score was significantly lower and percentage change significantly greater at 3months; global assessment of disease severity score: overall improvement at 3 months; physical function: significantly higher scores at 1 and 3 months	New Zealand
2004	Manicourt	RCT[1]	40	7 ±2 months	Alendronate vs. placebo	oral	VAS Pressure tolerance, edema and joint mobility	Significant decrease in mean VAS Increase in mean pressure tolerance and joint mobility	Belgium

Table 1 (continued)

Year	Author	Type	Sample size	Duration of disease (mean)	Medication	Route	Primary outcome measure	Outcome	Country
2008	Breuer	OL	10	4.3 ±3.1 years	Ibandronate	iv	Brief pain inventory, neuropathic pain scale patient's global impression of change scale	Patient global impression of change: 4 much improvement, 6 minimally improved; brief pain inventory: improvement; neuropathic pain qualities (9 of 10) and average and worst pain levels improved significantly	USA
2009	Santamato	CR	1	2 months	Clonadrate	im	Pain level with VAS	Great improvement	Italy
Immunoglobulin									
2002	Goebel	OL	11 of 130	>3 months	Immunoglobulin	iv	Ratio average pain intensity (API) value after or before therapy	20%: > 70% pain relief; 27.7%: pain reduction 25-70%; 4.6%: moderately increased pain levels, returned to pretreatment levels; rest: no effect, or pain reduction < 25%	Germany
2005	Goebel	CR	1		Immunoglobulin	iv		> 50% pain reduction	UK
2010	Goebel	RCT	13	6-30 months	Immunoglobulin vs. placebo	iv	pain intensity	Average pain intensity was 1.55 units lower	UK

CR= case report
OL= open label
RCT= randomized controlled trial
RCT[1]= RCT followed by open label
iv= intravenous
ivrb= intravenous regional block
im= intramuscular
VAS= visual analogic scale
PVS= pain verbal score
QST= quantitative sensory testing
ROM= range of motion

of the medication showed an additional effect; however, the difference was not significant.[60-62]

Side-effects were minimal (e.g. transitory flu-like symptoms); 1 patient dropped-out of one of the trials due to upper gastrointestinal intolerance.[60] No serious adverse events were described.

Immunoglobulin

The search yielded 1 case report, 1 open-label study, and 1 double-blinded RCT.

In the case report the patient recorded more than 50% pain reduction, accompanied by cessation of autonomic signs.[19] In the open-label study, only 11 of the 130 described patients were suffering from CRPS,[64] in the total group of patients, 20% had more than 70% pain relief, and 27.7% reported pain relief ranging from 25 to 70% relief.

The RCT was a double-blind, randomized, placebo-controlled study.[65] Patients received either the intervention in the first period and placebo in the second, or placebo in the first period and the intervention in the second. Pain intensity was the primary outcome measure and was 1.55 units lower after treatment with immunoglobulins compared with placebo. The treatment was associated with very few adverse events, except for moderate or severe headache and transient pain increase. No serious adverse events were reported.

DISCUSSION

This literature review was conducted to assess empirical evidence for the efficacy of various immunomodulating medication in CRPS patients.

The assessment is complicated by the fact that the cited studies show extensive methodological variability, that is, presence or absence of a control group, use of different designs, and varying sample compositions, diagnostic criteria and primary outcome measures. The exact impact of the outcome is often unclear.

The CRPS criteria applied for diagnosis vary between studies. The most common criteria are the IASP criteria[66], a revision of the criteria set has been proposed for both diagnostic and research purposes.[67] Because different criteria for diagnosing CRPS were used in the studies in this review, it is unlikely that all patients in these studies are comparable.

The studies covered the treatment of both acute and chronic conditions. A scintigraphic study to investigate whether inflammatory characteristics are present showed significantly more patients with early CRPS (existing for ≤ 5 months) with a positive scintigraphy compared with patients who had CRPS for a longer period.[7] Also, although the presence of local inflammation was confirmed in the first 2 years of CRPS, cytokine levels

did not correlate with either the characteristics or duration of the disease.[10] Therefore, the acute versus chronic classification is probably inadequate, and the time factor thus becomes less important.

It seems difficult to determine the appropriate period for treatment with immunomodulating medication. It seems more important to determine in each patient whether or not there is still an (ongoing) inflammatory process.

In addition, different primary outcome measures were used in the studies. In none of the studies was an improvement in inflammation measured. We suggest that a selection of 2 or 3 representatives from the inflammatory cytokines panel, the Th1/Th2 cytokines panel and the chemokines panel would be sufficient to indicate the activity of the CRPS disease; during the course of the disease this selected panel could also be used to indicate the effectiveness of therapeutic intervention.[13] This might allow to better determine which patients are likely to benefit from treatment with immunomodulating drugs.

Because the studies have different designs, the degree of empirical evidence yielded also differs. Most of the included articles were case reports or uncontrolled open-label studies. On the basis of these studies, TNF-α antagonists and thalidomide were reported to have a positive effect. Noteworthy, an open-label study, in which CRPS patients received lenalidomide (a thalidomide analog), showed that lenalidomide's pain and functional improvement sustained over 52 weeks of treatment. There would be some serious adverse events, suspected to be related to lenalidomide. However, this study only appeared in a poster presentation at a congress, and these results have not been published.[68]

The immunoglobulins were also investigated by means of a randomized double-blind placebo controlled trial; this trial also showed a positive effect, albeit a small one. However, for the glucocorticoids and bisphosphonates more RCTs have been performed. The glucocorticoids yielded 5 RCTs, of which the 2 blinded RCTs showed no benefit. However, a disadvantage is that the intervention in these 2 latter studies was administered by means of a Bier block, or intrathecally. In contrast, in the non-blinded trials, the oral glucocorticoids had a positive effect. Oral and intravenous bisphosphonates also appeared to have a positive effect. In our opinion, the use of bisphosphonates can be recommended; however, which medication, which dose, and for how long remains unclear. Our recommendation is in contrast to another group who also reviewed the 4 RCTs of bisphosphonates,[69] they concluded that, although bisphosphonates have the potential to reduce pain, there is insufficient evidence to recommend their use.

In summary, there is increasing evidence to show that inflammation does play a role in the pathophysiology of CRPS. Immune involvement brings a mechanism-based treatment within reach. On the basis of the results of this review, the use of immunomodulating medication may counteract the ongoing inflammation and might be an important

step in the recovery of the disabled hand or foot. However, as might be evident from the studies described above, this literature is of a very poor quality. Therefore, there is a need for more high-quality intervention studies.

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Chapter 5

Report of a preliminary discontinued double-blind, randomized, placebo-controlled trial of the anti-TNF-α chimeric monoclonal antibody infliximab in complex regional pain syndrome

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ABSTRACT

Objective

Inflammation appears to play a role in CRPS as, for example, cytokines (like TNF-α) are involved in the affected limb. The ongoing inflammation is probably responsible for the central sensitization that sometimes occurs in CRPS. Thus, early start of a TNF-α antagonist may counteract inflammation, thereby preventing rest damage and leading to recovery of the disease.

Design

Patients (n=13) were randomly assigned to infliximab 5mg/kg or placebo, both administered at week 0, 2, and 6.

Outcome measures

The aim was to confirm a reduction in clinical signs of regional inflammation (based on total impairment level sumscore: ISS) after systemic administration of infliximab. Also, levels of mediators in the fluid of induced blisters were examined in relation to normalization and improvement in quality of life.

Results

Six patients received infliximab and 7, placebo. There was no significant change in total ISS score between the two groups. Similarly, no significant difference in change in cytokine levels was found between infliximab compared to placebo. However, there was a trend toward a greater reduction of TNF-α in the intervention group compared to the placebo group. A subscale of the EuroQol (ie EuroQol VAS) revealed significant decrease in health status in the intervention group compared with the placebo group.

Conclusions

This study was terminated before the required number of participants had been reached for sufficient statistical power. Nevertheless, a trend was found toward an effect of infliximab on the initially high TNF-α concentration.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a disabling disease that usually occurs as a complication in an extremity after trauma or surgery. However, spontaneous development is also described. CRPS is characterized by spontaneous pain, allodynia, and hyperalgesia. The pain is disproportionate to the precipitating injury. Additional clinical signs include edema, disturbed blood flow to the skin or abnormal sudomotor activity, motor dysfunction, and trophic changes in the affected limb. The clinical picture was first described more than 100 years ago by Sudeck. Based on a consensus meeting of the IASP in 1993, the term 'complex regional pain syndrome' has been agreed upon.[1]

The estimated incidence ranges from 5.46 to 26.2 per 100,000 person-years. CRPS in adults most often occurs in the upper extremities, and a fracture is the most common initial event. Women are affected 3.4-4 times more often than men. The mean age at diagnosis is similar in men and women and ranges from 47 to 52 years.[2]

The acute phase appears to be a disorder of exaggerated neurogenic inflammation with increased skin temperature, edema, redness, pain, and loss of function (the so-called warm dystrophy).[3] These signs and symptoms may be part of the normal physiological response of the body after a trauma and in most patients resolve after a while. In CRPS patients, however, they may even become aggravated. This is suggestive of an insufficient remission of inflammation in CRPS, which may be one of the initial pathogenic factors. There is evidence of a pro-inflammatory profile in CRPS with an increase in neuropeptides, cytokines, and other mediators of inflammation[4-10], which may induce pain and hyperalgesia by direct and indirect mechanisms. The concept of a pathophysiological role of cytokines in CRPS is further supported by reports of successful treatment for CRPS with an anti-TNF agent, infliximab.[11]

The possible mechanism of action of anti-TNF agents are inhibition of inflammatory 'cytokine cascade' mediated by TNF; sequestration of TNF by binding; complement-mediated lysis of cells expressing TNF; altered leukocyte recruitment and endothelial activation; reduction of vascular endothelial growth factor expression and neovascularization; restoration of function of regulatory T cells, and induction of T lymphocyte apoptosis.[12]

Furthermore, continuing inflammation is probably responsible for central sensitization and neuroplastic changes in the spinal cord and higher centers of the central nervous system, expressing itself in allodynia, hyperalgesia, autonomic nervous system disturbances, and dystonia.[9]

The use of anti-TNF might counteract the ongoing inflammation and be an important step toward restoring the mobility of the disabled hand or foot, leading to further recovery of the disease. Given in an early stage, anti-TNF may also prevent the central changes in the nervous system, thereby reducing a change on rest damage.

The main aim of the present study was to test the hypothesis that systemic administration of infliximab would reduce the clinical signs of regional inflammation in the CRPS hand or foot. The secondary aims were improvement in the subjective scores of quality of life and normalization of the levels of inflammatory mediators in fluid of induced blisters.

The trial was registered as ISRCTN75765780.

This study is an investigator-initiated study performed by our research group, that is, Center for Pain Medicine of the Erasmus Medical Center, sponsored by Centocor, Inc. The company gave a grant for the drug and placebo. Centocor, Inc. was in no way involved in the design, performance and analysis of the study, and writing of this article. Performing the study, we had a serious recruitment problem that resulted in the decision of Centocor, Inc. to stop the sponsoring of the study. Unfortunately, the costs of the drug and placebo were too high to continue independently. Despite the early discontinuation, we think that it is important to share the available data with the scientific world. Those data are important for new insight in the pathophysiology and the design of new studies. Moreover, we think it is unethical to the patients who participated in the study not to make use of those data.

METHODS

The goal was to include 24 patients with CRPS in one extremity, recruited from a number of collaborating pain treatment clinics in the south-western part of the Netherlands and enrolled in one center in Rotterdam.

The diagnosis of CRPS was confirmed according to the IASP criteria.[13] The following were inclusion criteria: (1) patients should have untreatable inflammatory signs during recently developed CRPS (> 3 months and <12 months after the initial trauma), (2) they should be considered eligible according to the tuberculosis screening criteria set, (3) patients should have no previous administration of infliximab and should not be using drugs which suppress formation of inflammatory mediators, such as corticoids and nonsteroid anti-inflammatory drugs (NSAIDs).

The included patients were randomly assigned in a sequential fashion to one of the following treatment groups: Group I infliximab (5 mg/kg) in saline solution (0.9%); Group II placebo as saline solution (0.9%). Randomization was done in a 1:1 ratio in blocks of 4 patients for active and placebo treatments, respectively. The randomization was performed by the clinical pharmacy department of the Erasmus Medical Center using a computer generated random list. This department also prepared the drugs. Infliximab or placebo was administered intravenously at weeks 0, 2 and 6. Both groups received physiotherapy once a week, as well as instructions for personal daily exercise.

The protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center Rotterdam (MEC no. 2004-341) and the study was conducted according to the Guidelines of the Declaration of Helsinki and for Good Clinical Practice.

The primary outcome parameter was the reduction in clinical signs of regional inflammation in the CRPS hand or foot. The secondary outcome parameters were improvement in the subjective scores of quality of life and normalization of the levels of inflammatory mediators in fluid of induced blisters.

Evaluation of change in regional inflammation

To assess the effect on regional inflammation, the total impairment level sumscore (ISS)[14] was measured. The ISS almost completely reflects the five typical signs of inflammation, that is, redness, swelling, increased temperature, pain, and dysfunction. The evaluation was performed at screening (visit 1) and at week 10 (study end = visit 10).

Edema (volume by displacement)

Difference in volume was assessed by a volumeter (measuring the displacement of fluid by immersion of a body part). The difference between the involved and contra-lateral extremity was calculated as a percentage of the contra-lateral extremity. Normal asymmetry in volume should be less than 2% between both extremities.

Difference in skin surface temperature (videothermography)

Temperature difference between the involved and contra-lateral extremity was measured by computerized videothermography. This resulted in a mean difference and a calculated asymmetry factor. A normal asymmetry factor should be between 0.90 and 1.00.

Pain (VAS)

The amount of pain was measured with a visual analogue scale (VAS) in millimeters (range 0-100, from no pain to most intense pain).

Pain intensity (McGill Pain Questionnaire)

Pain intensity was evaluated with the McGill Pain Questionnaire Dutch Language Version (MPQ-DLV). The MPQ is the gold standard against which other measures of pain are tested. Patients are presented with 80 adjectives in groups, and they select one from each group that most closely matches their own pain. The weighted scores are added to produce a total score.

Mobility (active range of motion)

Mobility was measured by the difference in the active range of motion (AROM) of predefined joints in the involved and contra-lateral extremity. AROM is defined as the arc of

motion with muscle power to achieve the motion of a joint. In the upper extremity, the AROM is measured for the dorsal/palmar flexion of the wrist and for the flexion/extension in the metacarpophalangeal and proximal interphalangeal joints of the two most restricted digits. In the lower extremity, the AROM is measured for the flexion/extension in the knee, ankle, and digit 1 of the foot and the inversion and eversion of the ankle.

Inflammatory mediators in skin blister fluid

Infliximab directly affects local levels of TNF-α and may therefore influence the TNF-α-induced process. To determine whether this occurs, levels of TNF-α were measured in skin blister fluid. Suction blisters were made on the involved and contra-lateral extremities.[9] The assessment was performed at screening and at week 10.

Other mediators were also measured. A selection of two or three representatives from the inflammatory cytokines panel, the Th1/Th2 cytokines panel, and the chemokines panel would be sufficient to indicate the activity of the CRPS disease.[15]

Quality of life (EuroQol, SF-36 Health Survey questionnaire, SCL-90)

To assess the effect of treatment on quality of life, the EuroQol, SF-36 and SCL-90 questionnaires were used. The assessment was performed at screening and at week 10.

Safety evaluation

Safety was evaluated based on adverse events (reported and observed) and on clinically significant changes in laboratory values (chemistry profile, and complete blood count including differential and platelet counts).

Statistical analysis

Sample size consideration. Based on our data in 46 patients[9,10,16] we estimate that the screening value of the ISS will be 37.0 with a standard deviation of 5.3. Assuming independency of the standard deviation of the group differences with by SQRT 2 times the standard deviation of the baseline assessment, considering a significance of 0.05 and a power of 80%, and an expected difference of 10% between the pre- and post-treatment measurement in the placebo group and an expected difference of 40% between the pre- and post-treatment measurement in the infliximab group, 24 patients (2 x 12) should be included in the study.

All continuous variables were summarized by including the number of observations, mean, standard error of the mean (SEM), and the median. The relation between two dichotomous variables was tested using Fisher's exact test (two-sided). Differences between the affected side and the contra-lateral side within a specific group were tested using the Wilcoxon Rank Test.

Because of the small number of patients, the efficacy of infliximab was evaluated by calculating for all endpoint variables, the change in scores from visit 1 (screening) to visit 10 (end of study) in both groups. Change in the intervention group compared with the placebo group was evaluated using the nonparametric test for independent samples (Mann-Whitney U-test, two-sided).

RESULTS

Patients were included between January 2006 and December 2007. After 2 years, the recruitment was stopped; many otherwise eligible patients had an exclusion criterion (e.g. use of NSAIDs) or many declined to participate (mainly due to fear of side effects).

Finally, 13 patients with CRPS participated: 6 in the intervention group and 7 in the placebo group (Figure 1; CONSORT Diagram). All patients had continuing pain, as well as a history of hyperesthesia, asymmetry of skin color, edema and dysfunction. All patients fulfilled the IASP criteria. Demographic and disease-related baseline characteristics showed no significant difference between the two groups. Table 1 presents these patient characteristics.

Table 1. Characteristics of the study population

Patient characteristic	Intervention	Placebo
Gender: male/female	0/6	0/7
Mean age in years (range)	43.75 (25.15-56.87)	52.71 (34.33-70.94)
Affected extremity: arm/leg	4/2	4/3
Affected side: right/left	4/2	2/5

Total ISS

The change in volume, and difference in skin temperature and/or AROM between the involved and the contra-lateral extremity showed no significant difference between the two groups. Also, for pain intensity there was no significant decrease in or between the two groups. Pain scores (VAS) were found to decrease between visit 1 and visit 10 in both groups. However, the decrease in current pain did not significantly differ between both groups.

Thus, in these patients, there was no significant decrease in total ISS in the intervention compared with the placebo group. This result is complicated by the fact that the data concern (in total) eight arms and five legs.

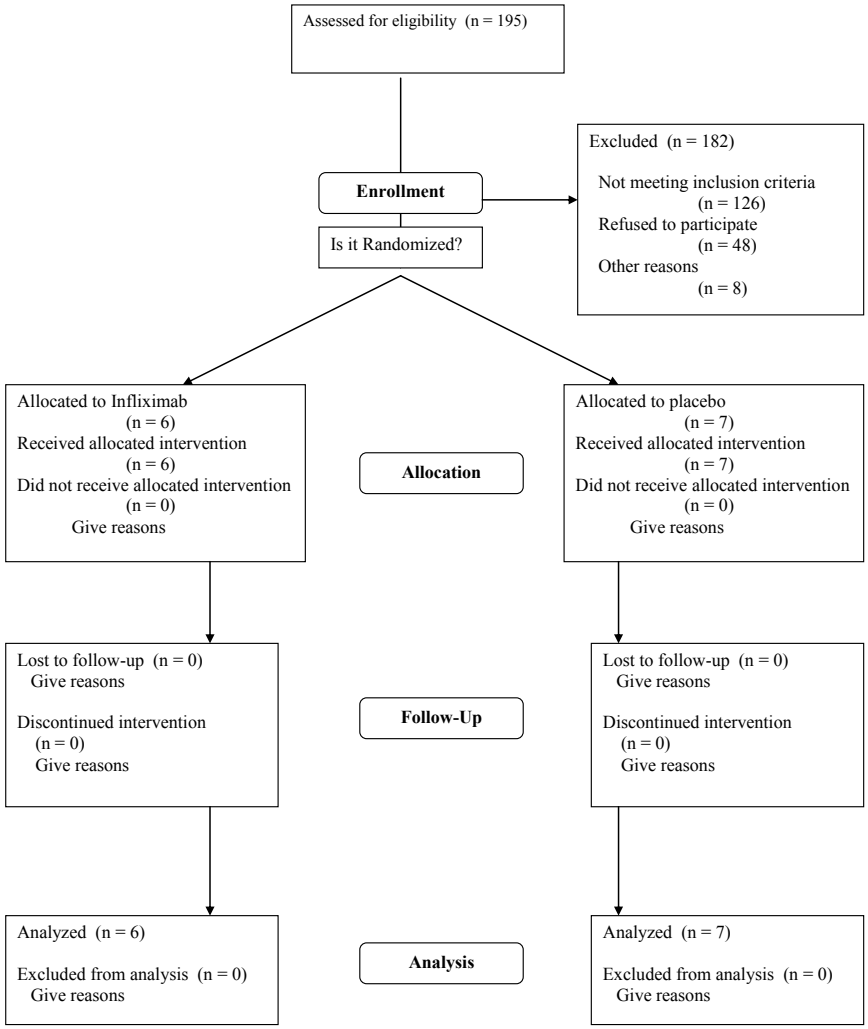

Figure 1. CONSORT: anti-TNF-α in CRPS

Inflammatory mediators in skin blister fluid

Table 2 and Figure 2 present data for all participants on cytokine levels of the involved vs. the contra-lateral extremity as measured at visit 1 and visit 10.

At visit 1, the levels of three cytokines in the involved extremity were compared with the contra-lateral extremity, that is, TNF-a (pg/ml) ($Z = -2.578$, $P = 0.10$), IL-8 (pg/ml) ($Z = -2.667$, $P = 0.008$), and Rantes (pg/ml) ($Z = -2.312$, $P = 0.02$). In addition, another cytokine showed a trend toward a higher level in the involved extremity at visit 1 compared with visit 10, that is, IP-10 (pg/ml) ($Z = -0.778$, $P = 0.08$).

The effect of the intervention was analyzed by testing the difference in cytokine levels between the placebo and intervention group at visit 10 compared with the differ-

Table 2. Mediators of inflammation

		Involved extremity				**Contra-lateral extremity**			
		Visit 1 (Screening)		Visit 10 (End of study)		Visit 1 (Screening)		Visit 10 (End of study)	
Cytokine		Intervention	Placebo	Intervention	Placebo	Intervention	Placebo	Intervention	Placebo
TNF-a (pg/ml)	Mean	40.32	67.05	1.94	30.07	31.84	39.78	1.88	29.12
	Sem	14.21	9.86	0.26	5.89	8.68	8.15	0.33	7.47
	Median	30.8	62.2	2.2	28.9	26.9	38.3	2.2	23.85
IL-2R (pg/ml)	Mean	115.45	179.5	157.48	164.07	69.48	147.65	159.38	231.33
	Sem	19.89	33.08	61.19	57.19	14.13	39.61	77.82	79.15
	Median	109.05	183.6	118.0	109.05	61.1	117.65	116.5	197.5
IL-6 (pg/ml)	Mean	48.43	60.98	93.64	39.78	30.3	52.43	88.53	116.6
	Sem	30.16	21.23	65.59	11.13	15.15	21.62	55.63	54.83
	Median	21.05	45.6	24.8	44.3	11.1	29.9	50.75	97.4
IL-8 (pg/ml)	Mean	475.37	781.28	688.72	243.77	219.88	406.82	697.4	294.22
	Sem	320.1	334.93	311.9	77.41	113.80	201.62	273.9	73.29
	Median	208.8	445.35	250.9	200.7	144.7	212.75	713.05	311.15
IP-10 (pg/ml)	Mean	136.15	124.1	141.12	121.67	108.8	108.6	91.43	125.4
	Sem	13.72	30.08	47.69	39.55	28.84	73.46	46.6	142.2
	Median	135.75	102.65	77.6	83.55	92.5	92.5	58.3	68.95
RANTES (pg/ml)	Mean	25.02	32.2	44.94	20.95	18.9	23.73	82.08	21.58
	Sem	2.98	7.13	22.23	1.52	0.707	2.57	61.21	1.50
	Median	24.95	23.2	23.7	22.8	18.9	21.8	21.85	22.3

Sem = standard error of the mean

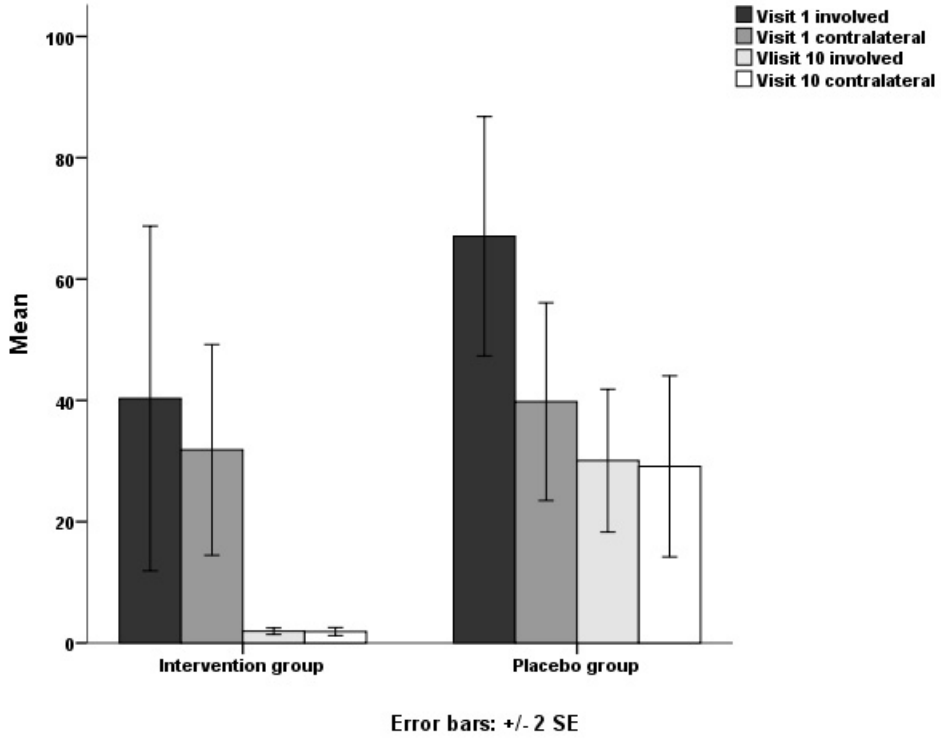

Figure 2. TNF-α (pg/ml) at screening and at end of the study by side and by study group

ence in levels at visit 1. No significant differences were found in the change in cytokine levels between the two groups. However, a trend was found toward a larger reduction in the level of TNF-a (pg/ml) in the intervention group compared with the placebo group (P = 0.07).

Quality of life (EuroQol, SF 36 Health Survey questionnaire, SCL-90)

In the intervention group, the EuroQol VAS score at visit 1 was increased compared with that at visit 10, whereas in the placebo group the effect was neutral; this difference in change over time between the two groups was significant (P = 0.03) (Table 3). In other words, compared to placebo the health status in the intervention group became significantly worse.

For the remaining EuroQol subscales scores, no significant differences over time were found between the groups. Furthermore, no significant difference (or a trend toward it) was found in the scores of the other outcome parameters.

Table 3. EuroQol subscale scores

Subscale		Visit 1 (Screening)		Visit 10 (End of study)	
		Intervention	Placebo	Intervention	Placebo
Mobility (walking)	No problems	4 (66.67%)	4 (57.14%)	3 (50.0%)	4 (57.14%)
	Some problems	2 (33.33%)	3 (42.86%)	3 (50.0%)	3 (42.86%)
	Bedridden	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Self care (wash/ dress yourself)	No problems	3 (50.0%)	2 (28.57%)	2 (33.33%)	3 (42.86%)
	Some problems	3 (50.0%)	5 (71.43%)	4 (66.67%)	4 (57.14%)
	Unable	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Daily activities	No problems	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Some problems	5 (83.33%)	7 (100%)	6 (100%)	7 (100%)
	Unable	1 (16.67%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Pain or other complaints	No	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Moderate	3 (50.0%)	5 (71.43%)	4 (66.67%)	5 (71.43%)
	Very severe	3 (50.0%)	2 (28.57%)	2 (33.33%)	2 (28.57%)
Mood (Anxious or depressed)	No	5 (83.33%)	4 (57.14%)	4 (66.67%)	5 (71.43%)
	Moderately	1 (16.67%)	2 (18.57%)	1 (16.67%)	2 (28.57%)
	Severely	0 (0.00%)	1 (14.29%)	1 (16.67%)	0 (0.00%)
EuroQol-5D	Mean (SEM)	0.40 (0.14)	0.41 (0.10)	0.43 (0.16)	0.50 (0.10)
	Median	0.41	0.59	0.66	0.66
EQ-5D VAS score*	Mean (SEM)	71.67 (7.82)	50.71 (2.77)	45.33 (15.3)	62.43 (7.72)
	Median	75	50	46.5	50

* Difference in EQ-5D VAS score visit 10 vs. visit 1; P = 0.03
SEM = standard error of the mean

Safety evaluation

During this trial, one serious adverse event was reported. This concerned a patient in the placebo group who was involved in a car accident four months after completion of the study. She suffered multiple fractures and was hospitalized, followed by treatment in a rehabilitation center. The relationship to the treatment was classified as 'other'. No serious adverse events were reported in the intervention group.

Registration of all other adverse events revealed 28 in the infliximab group and 51 in the placebo group. Examples of adverse events in the infliximab group are one patient with increased blood pressure during infusion, and headache, dizziness, diplopia, and nausea in the days thereafter; another patient reported constipation, a light headache, malaise, flu-like symptoms, and episodes with a herpetic lip during 1 year; and one patient reported flu-like symptoms.

DISCUSSION

From the start of the study, there were problems with recruiting patients. We tried to improve this by collaborating with anesthesiologists throughout the south-western part of the Netherlands, by giving lectures to patient organisations and to the Dutch CRPS patient organisation, by advertising on the website of the patient organisation, and by distributing newsletters to family doctors, neurologists and anesthesiologists in the region.

We assume several reasons why so few patients could be included. First, after publication of the Dutch CRPS guidelines in 2006, many family doctors and peripheral specialists have the tools to treat acute CRPS patients (e.g. with vitamin C, fluimucil or DMSO). We clearly see a decline in the referrals after the publication of this guideline. Only patients who did not respond to this initial therapy were later referred to our clinic to (perhaps) participate in this trial. Although we tried to minimise the delay before the visit to the clinic, there were always more complicated/chronic CRPS patients available than acute CRPS patients. Many of these sub-chronic patients exceeded the inclusion criterion of a maximum of 12 months after the initial trauma.

Several eligible patients declined to participate: some because they considered placebo treatment to be a risk and others because they expected their symptoms to improve without the offered medication.

On reflection, excluding patients using NSAIDs is perhaps too academic. These drugs were not allowed because they might suppress formation of inflammatory mediators. However, these drugs affect prostaglandins and not cytokines like TNF-α.

In this relatively small study, we found an indication for effect on the inflammatory mediator TNF-α and a significant deterioration on a subscale of quality of life, but not on the other parameters. We cannot explain this deterioration. It could be an accidental finding by multiple testing or because of sampling variation.

There was an interesting decrease in the inflammatory mediator. Figure 2 shows that, at screening, TNF-α is higher on the involved side than on the contra-lateral side. This is in accordance with our earlier work[9-11, 17] and was the rationale for this study with infliximab. After infliximab, TNF-α was reduced to less than 2 pg/ml. TNF-α was also reduced in the placebo group, but to values of about 30 pg/ml. It is unclear what caused this reduction; perhaps the initial inflammation reduced during the natural course of the disease.

In the acute phase of CRPS neuroinflammation occurs, where free radicals inflict damage on several local tissues.[18,19] Furthermore, changes in neuroplasticity and central sensitisation have been described. Thus, even when the inflammation has been controlled by anti-TNF, the damage to local tissue[20-22] and the changes in neuroplasticity and central sensitization still persist.

Following this thought, it makes sense to start treatment of CRPS as early as possible to minimize the complications of this devastating disease. However, this might explain the restricted effect of anti-TNF on (most) of the outcome parameters.

Another limitation is the inclusion of patients based on a criteria set of physical symptoms. It is known that CRPS can express itself in more than one extremity. In this study, we make use of the contra-lateral extremity as control. It is unlikely, however we cannot definitely exclude influence of this phenomenon. Maybe it would be more appropriate to include only those patients with a high concentration of inflammatory mediators in the involved extremity and normal values in the contra-lateral extremity, especially TNF-α.

Definite conclusions are difficult, on one hand due to lack of power, and on the other hand due to the increased level of type 2 error as a result of multiple comparison, which might have lead to incorrectly rejecting the null-hypothesis of no difference.

Therefore, a larger follow-up study on the effects of TNF-α antagonists on inflammation in CRPS patients is recommended. Hopefully, this contributes to a better understanding of the mechanism of interactions in CRPS. However, considerations should be made on the selection criteria for inclusion of patients; the primary outcome measure should be considered as well. One might well consider whether the choice for inflammation was ideal.

In spite of the small study group, a promising trend was found toward an effect of infliximab on the initially high TNF-α concentration in patients with neuroinflammation in acute CRPS. Infliximab appears to be a safe treatment for patients with regional inflammation in the acute stage of CRPS. Unfortunately, because this study was terminated before the required number of participants for sufficient power had been reached, we cannot draw any definite conclusions.

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Chapter 6

Mast cells: a new target in the treatment of complex regional pain syndrome?

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ABSTRACT

There is convincing evidence that inflammation plays a pivotal role in the pathophysiology of complex regional pain syndrome (CRPS). Besides inflammation, central sensitization is also an important phenomenon. Mast cells are known to be involved in the inflammatory process of CRPS and also play a role (at least partially) in the process of central sensitization.

In the development of a more mechanism-based treatment, influencing the activity of mast cells might be important in the treatment of CRPS.

We describe the rationale for using medication that counteracts the effects of mast cells in the treatment of CRPS.

INTRODUCTION

Complex regional pain syndrome (CRPS) is a painful and disabling complication after surgery or physical trauma, but spontaneous development is also described.

A careful clinical evaluation of signs and symptoms remains the cornerstone of CRPS diagnosis. The general acceptance of the IASP diagnostic criteria for diagnosing CRPS has contributed to improved clinical communication and the ability to generalize research findings.[1]

The estimated overall incidence rate of CRPS is 26.2 per 100,000 person-years.[2] Females are affected at least three times more often than males. The upper extremity is affected more frequently than the lower extremity, and a fracture is the most common precipitating event (44%). Severe CRPS outcome is rare, but a majority of patients still suffer from persistent impairments two or more years post onset.[3]

It is reasonable to assume that different mechanisms are involved in a complex network of interactions, resulting in the painful and impairing disorder of CRPS.[4] There is evidence that inflammation is one of the mechanisms that play a role in the pathophysiology of CRPS. The contribution of inflammation is also underlined by the successful reports from studies on treatment with immunomodulating agents, recently reviewed.[5]

The use of immunomodulating medication may counteract the ongoing inflammation; early use may be an important step in preventing sensitization. Therefore, such treatment may play an important role in recovery of the disabled hand or foot. However, it is unknown whether (apart from the immunomodulating medication) other drugs might also counteract the inflammation. Based on empirical findings on the role of mast cells in the pathophysiology of inflammation in CRPS[6], we describe the rationale for the use of medication targeting mast cell activity.

PATHOPHYSIOLOGY OF CRPS

Complex regional pain syndrome often displays the classic aspects of inflammation.[7] Inflammation contributing to CRPS can arise from two sources.[8] First, classic inflammatory mechanisms can contribute through actions of immune cells (such as lymphocytes and mast cells), which, after tissue trauma, secrete pro-inflammatory cytokines including interleukin-1β, -2,-6, and tumor necrosis factor (TNF)-α. Secondly, neurogenic inflammation may also occur, mediated by release of pro-inflammatory cytokines and neuropeptides directly from nociceptive fibers in response to various triggers. Neuropeptide mediators involved in neurogenic inflammation include substance P, calcitonin gene-related peptide, and bradykinin (which is also involved in initiating cytokine release).

Over time, various studies have report the role of inflammation in the pathophysiology of CRPS. In venous blood of patients with CRPS elevated mRNA levels of pro-inflammatory cytokines, TNF and IL-2 have been found, as well as a reduction of mRNA levels of the anti-inflammatory cytokines IL-4 and IL-10.[9] However, local rather than systemic inflammatory responses appear to be relevant in CRPS.[10] No significant changes in serum parameters in CRPS were found compared with control groups, indicating the local formation of mediators. The presence of a local inflammation was already suspected based on evidence found in a scintigraphic study on CRPS, which demonstrated vascular permeability for macromolecules.[11] Technetium 99m-anti-TNF-α antibody scintigraphy revealed that TNF-α was only localized in the affected hand of patients with early CRPS.[12]

Increased levels of pro-inflammatory cytokines have been detected in fluid from artificially raised skin blisters in the involved extremity in comparison with the contra-lateral site; however, no correlation was found between levels of pro-inflammatory cytokines and the characteristics or duration of the disease.[13-15] Using multiplex bead array assays to establish the involvement of cytokines in inflammatory processes revealed 10 detectable representative cytokines in blister fluid of CRPS patients.[16] This finding suggests that these mediators were generated by a homogenous cell population. Because T-cells are apparently not involved, the most likely candidates are monocytes, macrophages and, possibly, fibroblasts and skin mast cells.

Mast cells are known to be involved in CRPS.[6] Tryptase is a specific proteolytic enzyme and a good marker of the presence of mast cells. Activated mast cells synthesize and release tryptase. Significantly higher levels of tryptase have been demonstrated in the involved extremity compared with the contra-lateral extremity. A significant correlation between levels of IL-6 and TNF-α in the involved extremity was observed, but no significant correlation was found between levels of tryptase, IL-6 and TNF-α. Also, a significant correlation was found between the reported pain intensity as measured on a visual analog scale (VAS) and tryptase levels in the involved extremity, but not between the VAS and IL-6 or between the VAS and TNF-α.[6]

Pro-inflammatory cytokines and neuropeptides are responsible for peripheral sensitization leading to increased nociceptive responsiveness. Persistent or intense noxious input resulting from tissue damage or nerve injury triggers increased excitability of nociceptive neurons in the spinal cord, a phenomenon called 'central sensitization'.[8] An objective phenomenon which is associated with central sensitization is windup, which is reflected in increased excitability of spinal cord neurons. CRPS patients display significantly greater windup to repeated stimuli.[8]

The anatomical proximity of mast cells to neurons in both the central nervous system (CNS) and peripheral nervous system, their migratory ability, and their ability to release potent vasoactive and inflammatory mediators, constitutes an important neuroimmune

axis.[17] The presence of 'cross-talk' has been shown between mast cells and cells of the CNS in various neurodegenerative diseases having an inflammatory and/or autoimmune component. The CNS interacts with the immune system by means of its neuropeptides, neurohormones, and neurotransmitters; in turn, the immune system feeds back to the brain which subsequently induces changes both in behavior (sickness response) and in the immune system.[18] Nerve plasticity and remodelling occur extensively during an inflammatory process, in that there is an increase in nervous innervation as well as mast cell density.

NEW PATHOPHYSIOLOGY-BASED PERSPECTIVES FOR PHARMACOLOGICAL INTERVENTION

Mast cells are bone-marrow-derived cells capable of secreting many active molecules: mediators stored in specific granules (such as histamine and heparin); small molecules produced immediately upon stimulation (such as nitric oxide); and many constitutively secreted, pleiotropic cytokines.[19] They play an important role in innate or acquired immunity, in bacterial infections, and also in autoimmunity.[20] A number of cytokines (e.g., IL-1, IL-6, TNF) are synthesized de novo and released several hours after stimulation.

Thus, because mast cells are known to be involved in inflammation in CRPS[6], it can be assumed that controlling these cells might (in part) improve inflammation in CRPS.

Counteracting the effects of the mast cell can be achieved by the following: (1) prevention the division of, or killing the cell, (2) prevention of release, or (3) use of anti-TNF/anti-IL 6 therapy when TNF-α/IL-6 is released.

Prevention of division/killing the mast cell

This effect is achieved by means of tyrosine kinase inhibitors. They inhibit the intrinsic tyrosine activity of several specific proteins, including KIT.[21] KIT is a transmembrane protein expressed on a variety of cells, including mast cells. In mast cells, KIT acts as a receptor for the stem cell factor. Binding of the stem cell factor on KIT is essential for the survival, differentiation, chemotaxis, and functional activity of mast cells. Thus, inhibition of KIT results in decreased mast cell population and activity.

Inhibition of the intrinsic activity of several proteins also results in reduced tumor vessel growth or carcinogenesis. Examples include imatinib (Glivec), which became clinically available in 1998 for the treatment of patients with chronic myeloid leukemia in the chronic phase resistant to interferon-α[22] and sunitinib, an anticancer drug currently used in the palliative treatment of metastatic renal cell carcinoma and gastrointestinal stomal cell tumors.[21] As far as we know, these two drugs have not yet been used in CRPS.

Prevention of release from the mast cell

This effect can be achieved by means of 3 types of drugs.

The first type is glucocorticoids. Besides the fact that glucocorticoids are anti-inflammatory via a number of mechanisms[23], they also appear to affect mast cells degranulation.[24] They could rapidly inhibit IgE-mediated exocytosis and histamine release of mast cells. This effect is not accomplished by direct action on secretion machines, but is mediated by a reduction in the [Ca(2+)](i) elevation.

The second type is the anti-histamines. Cytokine production by human mast cells can be modulated (to some extent) by the H1-antagonists (e.g. azelastine, loratadine, and cetirizine) as well as by the H2-antagonist ranitidine.[25] Of the cytokines studied, TNF-α has been shown to be the most susceptible to inhibition by antihistamines. The exact mechanism of action of antihistamines on cytokine secretion from mast cells remains to be elucidated.

Finally, there is the cell stabilizer. Disodium cromoglycate is a cell stabilizer which inhibits the release of preformed and newly synthesized chemical mediators from a variety of cells involved in allergic and inflammatory responses. It is assumed that cromoglycate acts on the lipid bilayer membrane and thereby regulates the degranulation of mast cells by stabilizing membrane fluidity.[26]

Although the use of glucocorticoids in CRPS appears to have a positive effect, empirical evidence for their use is scarce.[5, 27] The mechanism by which this effect is achieved is not yet clear. Neither cromoglycate nor antihistamines have been used in the treatment of CRPS.

Use of anti-TNF therapy/anti-IL-6

If release of cytokines does occur, it might be useful to administer drugs which affect these cytokines.

Anti-TNF

* Tumor necrosis factor-α antagonists

Tumor necrosis factor alpha (TNF-α) is a cytokine which promotes the inflammatory response. The possible mechanisms of action of anti-TNF agents are, for example, inhibition of the inflammatory 'cytokine cascade' mediated by TNF, sequestration of TNF by binding, and complement-mediated lysis of cells expressing TNF.[28]

The effect of using this drug in the treatment of CRPS has been described in 2 case reports of 3 patients.[29, 30] All 3 patients showed improvement in pain, temperature, and motor function.

* Thalidomide

Thalidomide exerts a selective effect by suppressing TNF-α secretion only. It inhibits TNF-α production by human blood monocytes, without influencing either general protein synthesis or the expression of other monocyte-derived cytokines.[31]

In 2 case reports, thalidomide was introduced for CRPS patients with a comorbid condition.[32, 33] In both cases, there was a beneficial effect on CRPS. An open-label study, in which 42 patients were treated, has shown a "dramatic response" in 17% of the patients, and 14% experienced at least modest pain relief and/or showed some reduction in the need for concurrent medication.[34] No results for the remainder of the patients have been reported.

Anti-IL-6

Interleukin (IL)-6 is a pleitropic proinflammary cytokine that is produced by multiple cell types. IL-6 signal transduction is mediated by membrane-bound and soluble IL-6 receptors. Tocilizumab (TCZ) is a recombinant humanized anti-human IL-6 receptor monoclonal antibody. TCZ binds to both of these receptors and inhibits signalling via this route.[35] TCZ has been approved for the treatment of rheumatoid arthritis in patients who have an inadequate response to one or more TNF-α inhibitors. Until now, TCZ has not been used in CRPS.

SUMMARY AND CONCLUSION

Woolf and Decosterd advocated a form of pain treatment based on the mechanisms involved in the pathogenesis of pain.[36] The aim should be to identify in each patient which mechanisms are responsible for their pain and specifically target treatment to those mechanisms.

Following this recommendation, the treatment of CRPS should be based on knowledge of the pathophysiological mechanisms underlying this condition.

It is assumed that several mechanisms play a role in CRPS, one of which appears to be inflammation. Mast cells generate pro-inflammatory cytokines which are involved in the inflammatory process of CRPS. Also, central sensitization is common in CRPS. In general, mast cells appear to be involved in the cross-talk with the CNS; this process could play a role in central sensitization in CRPS.

It appears appropriate to correct the baseline inflammatory status to lower disease activity and, thus, lower production of pro-inflammatory cytokines. Treating the inflammatory component of CRPS might also prevent central sensitization. Therefore, following the recommendations of Woolf and Decosterd, it is reasonable to tackle the mast cell.

It is difficult to decide which of the above-mentioned strategies for modulating mast cell activity might be preferred. Therefore, in selecting a drug for study, it seems wise to take into account the safety profile, side-effects, ease of treatment, and the cost of the drugs associated with each of the options.

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Chapter 7

General discussion

Complex Regional Pain Syndrome (CRPS) is characterized by a continuing regional pain that is disproportionate in time or degree to the usual course of any known trauma or lesion. The pain is regional, not in a specific nerve territory or dermatome, and usually has a distal predominance. CRPS is also characterized by a variable progression over time.

Apart from pain, additional clinical signs include disturbed blood flow to the skin or abnormal sudomotor activity, motor dysfunction, and trophic changes in the affected extremity. Based on signs and symptoms in the individual patient, it is possible to classify subtypes of CRPS: 1) a relatively limited syndrome with vasomotor signs predominating, 2) a relatively limited syndrome with mainly neuropathic pain/sensory abnormalities, and 3) a florid CRPS syndrome similar to 'classic RSD' descriptions.[1]

The pathophysiology of CRPS is complex and still not completely understood. Different mechanisms are known to be involved in the pathophysiology of CRPS, including inflammation, hypoxia, dysregulation of the autonomic nervous system, and changes in the somatosensory system.[2] In addition to the convincing role for inflammation, there are arguments to support involvement of the immune system in the pathophysiology of CRPS. For example, CRPS shows a beneficial response to treatment in open-label studies on treatment with immunomodulating medication, such as infliximab[3] and immunoglobulin.[4] Furthermore, similar to other inflammatory diseases, CRPS displays a female predominance[5] as well as an association with distinct human leukocyte antigen (HLA) alleles.[6-8]

In the work presented in this thesis, various immunological aspects in CRPS have been examined with the aim to achieve more insight into both the pathophysiology of CRPS and the potential pharmaco-therapeutic consequences of its treatment.

With regard to inflammation, aspects of both the clinical features of inflammation and asymmetric levels of the pro-inflammatory cytokines (TNF-α and IL-6) in CRPS have been explored.

It is assumed that patients with warm CRPS suffer from inflammation and that the asymmetric level of cytokines reflects the presence of inflammation. In cold CRPS, inflammation is generally assumed not to be present.[2] However, some patients with cold CRPS patients have displayed full-blown symptoms of warm CRPS after successful treatment with vasodilatation therapy.[9] Our study showed that three patient groups, differing in asymmetrical level of temperature (e.g. warm, cold and intermediate), showed no significant differences in asymmetric levels of the pro-inflammatory cytokines TNF-α and IL-6. This is in accordance with the idea that (a subgroup of) patients with cold CRPS might still suffer from inflammation. This finding implies that we should question the clinical grouping of CRPS patients into cold versus warm CRPS. It might be more appropriate to differentiate between patients who have inflammation and those who do not. This could, in turn, have therapeutic consequences, i.e. patients with underlying inflammation may benefit more from a therapeutic intervention which influences the

ongoing inflammation, rather than therapy that aims to influence the clinical aspect (such as vasodilators for cold CRPS). Therapy focused on influencing the clinical aspects could be one of the reasons why clinical trials have failed to demonstrate the efficacy of many commonly used interventions in CRPS.

A particular challenge of this approach is that the test commonly used to confirm the presence of inflammation, i.e. determining the pro-inflammatory cytokine levels in fluid from artificial skin blisters, is a time-consuming procedure. Until now, this has restricted its use to the field of research and makes it almost impossible to apply in clinical practice. In CRPS, the development of a simple, easy-to-use and inexpensive diagnostic method to demonstrate inflammation is urgently required.

Furthermore, in an individual patient, more than one mechanism can play a role in the pathophysiology of CRPS at the same time. Therefore, the often applied approach of a single-mechanism intervention, both in research and in clinical practice, will probably be disappointing. This implies that, if in the case of cold CRPS there is vasomotor disturbances in addition to inflammation, then the patient will probably derive more benefit from a multi-mechanism targeted approach. Notably, the only multi-mechanism therapy applied nowadays for CRPS, is spinal cord stimulation. This latter therapy has a positive effect on both the somatosensory system and vasomotor disturbances.[10] It is one of the few therapies that has proven successful in CRPS.[11] Future research needs to focus on a multi-mechanism treatment regime for application in CRPS.

Based on an animal model of CRPS, Coderre and colleagues suggested that the inflammation in CRPS is a continuum, in which the vasomotor disturbance is a consequence of the inflammation.[12] This is another interesting idea, because the focus is no longer on the different subtypes of CRPS but rather on the continuum of inflammation.

Autoimmunity has been suggested as one of the underlying mechanisms in the pathophysiology of CRPS. We explored the potential role of systemic and/or organ-specific autoimmunity. To do this, the prevalence of CRPS patients with a positive test for antinuclear antibodies (ANA) and for anti-neuronal antibodies was compared with the prevalence in the healthy population. The ANA prevalence was significantly higher in CRPS patients compared to the healthy population; however, this did not apply for the prevalence of the anti-neuronal antibodies. Also, the immunofluorescence pattern of the anti-neuronal antibodies proved to be different in CRPS patients compared to the healthy population. In CRPS patients, the prevalence of ANA was more similar to that observed in patients with an autoimmune disease such as rheumatoid arthritis (also referred to as an auto-inflammatory disease) than that in patients with a classic systemic autoimmune disease (like systemic lupus erythematosus), with a much higher observed prevalence.[13] This is a relevant finding because it may affect the choice of treatment. For example, use of medication such as TNF-α antagonists is more successful in an auto-inflammatory disease than in the classic auto-immune disease.

In order to define CRPS as an autoimmune disease, then Witebsky's criteria for an autoimmune disease should be considered.[14] These criteria include: 1) demonstration of a specific antigen, 2) circumstantial evidence of an autoimmune or inflammatory disorder from clinical clues, and 3) reproduction of clinical features in recipient animals by passive transfer of putatively pathogenic antibodies. CRPS definitely meets the second criteria. There are indications that the first criterion is also met; however, this applies only to some patients and more research is needed to define the specific antigens involved. Injection of serum-IgG from a CRPS patient into groups of mice resulted in abnormal physical behavior and a significant reduction in rearing.[15] However, because these findings are not a reproduction of the clinical features, as needed for the third criterion, the proposal by Goebel and colleagues[16] to define CRPS as a novel kind of autoimmune disease does not seem correct. Based on the results from our study, CRPS seems to be an auto-inflammatory disease.

Immunomodulating medication reduces the manifestation of inflammation by influencing the mediators of inflammation, such a cytokines, neuropeptides, eicosanoids and amino acids. If one assumes that inflammation plays an important role in CRPS, then one would expect immunomodulating medication to be effective. In the Netherlands, CRPS patients are treated according to the Dutch guidelines for CRPS[17], i.e. for inflammatory signs and symptoms free-radical scavengers are advised, whereas immunomodulating medication is not advised. However, CRPS has shown a beneficial response to some immunomodulating medication.[3,4] Therefore, a literature review was conducted to assess the empirical evidence for the efficacy of administering the most commonly used immunomodulating medication (i.e. glucocorticoids, TNF-α antagonists, thalidomide, bisphosphonates and immunoglobulins) in CRPS patients. Unfortunately, none of the studies measured (an improvement in) levels of inflammation. Furthermore, evidence for the use of immunomodulating medication proved to be small; the exception to this was the use of bisphosphonates, for which all four double-blind randomized controlled trials showed a significant decrease of pain.[18-21] It is noteworthy that, in contrast to our findings, another research group concluded that there was insufficient evidence based on these four randomized trials.[22]

To test the assumption that TNF-α antagonists can reduce the manifestation of inflammation in CRPS, a double-blind randomized placebo-controlled trial was conducted. Unfortunately, this study was terminated before the required number of patients for sufficient statistical power had been reached. Nevertheless, the limited data showed a trend towards a greater reduction of TNF-α in the intervention group compared with the placebo group.

Another research group presented a case series of CRPS patients in which adalimumab (TNF-α antagonist) was used.[23] Their results suggest that TNF-α antagonists can

be potentially useful in some patients, but not in all. There seem to be responders and non-responders for this treatment in CRPS.

There are several possible reasons for this changing effect (responders vs. non-responders). It seems appropriate to include only those patients with a high concentration of TNF-α in the involved extremity compared to the contralateral side. In other words, inflammation must be confirmed and the use of signs and symptoms alone is not adequate. Further, continuing inflammation is probably responsible for central sensitization, which is a common feature in CRPS.[24] This means that even when the inflammation has been controlled by medication, the central sensitization still persists. If central sensitization is present, it probably better to start multimodal therapy instead of monotherapy. In other words, different mechanisms can play a role at the same time and must be identified (and also be treated).

Unfortunately, there is (still) insufficient evidence to justify normal use of TNF-α antagonists. However, due to the growing evidence for the involvement of TNF-α in inflammation in CRPS, the directions for an auto-inflammatory disease, and the effect of TNF-α antagonists in some patients, a new randomized trial seems warranted. Future CRPS research on anti-inflammatory medication and, more specifically anti-TNF, needs to establish predictors to identify responders versus non-responders to this kind of therapy.

Mast cells are known to be involved in the inflammatory process of CRPS[25] and also play a role in the process of central sensitization. Therefore, influencing the activity of mast cells seems to be another therapeutic option. We have examined the possibilities of such an approach. Further exploration of the role of mast cell in the pathophysiology and, consequently, new therapeutic interventions are also important items for future research.

In 1999, in an editorial published in Pain, Clifford Woolf advocated for a shift in pain medicine from a symptom-based treatment towards a mechanism-based treatment. This would make treatment more rationale and, hopefully, more successful.[26] However, for CRPS much work remains to be done.

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Chapter 8

Summary

Nederlandse samenvatting

SUMMARY

Chapter 1

The introduction describes the rationale for this thesis. The pathophysiology of CRPS is complex and still not completely understood. In addition to a convincing role of inflammation, there are a number of other arguments why an involvement of the immune system has been suggested in the pathophysiology of CRPS. Therefore, some immunological aspects were further explored with the aim to achieve more insight in both the pathophysiology and possible treatment options in CRPS.

Chapter 2

In cold CRPS, inflammation is generally assumed not to be present. However, there are reports of cold CRPS patients, treated with vasodilatation therapy, who subsequently displayed a warm CRPS. This chapter presents the results of a retrospective analysis to test the hypothesis that inflammation could be 'hidden' behind vasomotor disturbances. For that purpose, we defined three types of CRPS: cold CRPS, intermediate (neither cold nor warm) and warm CRPS. Of these patients the difference between the level of the pro-inflammatory cytokines Il-6 and TNF-α in the affected extremity and that in the contralateral extremity was determined. The bilateral difference of the level of these cytokines did not differ between patients with cold CRPS, intermediate CRPS or those with warm CRPS. From this finding, we conclude that inflammation may (also) be involved in (a subgroup of) patients with a cold CRPS.

Chapter 3

Autoimmunity has been suggested as one of the pathophysiologic mechanisms that may underlie CRPS. Screening for antinuclear antibodies (ANA) is one of the diagnostic tests, which is usually performed if a person is suspected to have a systemic autoimmune disease. Anti-neuronal antibodies are auto-antibodies directed against antigens in the central and/or peripheral nervous system. The aim of this study was to compare the prevalence of these auto-antibodies in CRPS patients with that the healthy population. We found that the prevalence of a positive ANA test is significantly higher in CRPS patients than in the healthy population. The prevalence of the anti-neuronal antibodies however did not deviate from that in the healthy population. These findings indicate that auto-antibodies may be involved in the pathophysiology of CRPS, at least in a subset of patients.

Chapter 4

Immunomodulating medication reduces the manifestation of inflammation by acting on mediators of inflammation. If inflammation is involved in the pathophysiology of

CRPS, immunomodulating medication in CRPS patients could be beneficial. This chapter reviews the current empirical evidence for the efficacy of administering the most commonly used immunomodulating medication (i.e. glucocorticoids, tumor necrosis factor-α antagonists, thalidomide, bisphosphonates, and immunoglobulins).

Chapter 5

In this chapter the results of a discontinued double-blind, placebo-controlled trial of the anti-TNF α chimeric monoclonal antibody (infliximab) in CRPS are presented. The aim of the trail was to evaluate the clinical signs of regional inflammation (based on total impairment level sumscore: ISS) after systemic administration of infliximab. In addition, levels of mediators in the fluid of induced blisters were examined in relation to normalization and improvement in quality of life. This study was terminated before, for a sufficient statistical power, the required number of participants had been reached. Nevertheless, a trend was found towards a greater reduction of TNF-α in the intervention group.

Chapter 6

Mast cells are known to be involved in the inflammatory process of CRPS and also to play a role in the process of central sensitization. In the development of a more mechanism-based treatment of CRPS, influencing the activity of mast cells might be important. This chapter describes the rationale for using medication that counteracts the effect of mast cells in the treatment of CRPS.

Chapter 7

In the general discussion, the focus of this dissertation is explicated. The current state of knowledge and theory concerning the role of inflammation in CRPS is summarized. In addition, the findings of the trials performed are enumerated and commented. It raises a shift in thinking about the concept of the pathogenesis of CRPS. Nowadays, the most accepted idea is that we are dealing with different phenotypes of CRPS. In this way it is explained that there is a clinical variability in expression of the disease between patients. Here, the concept of a continuing inflammatory process with variable secondary consequences is presented.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1

De pathofysiologie van CRPS is ingewikkeld en is nog niet volledig begrepen. Naast de overtuigende rol van inflammatie zijn er nog een aantal andere argumenten voor de betrokkenheid van het immuunsysteem. Een aantal immunologische aspecten zijn daarom verder onderzocht, met het doel meer inzicht te krijgen in zowel de pathofysiologie als de behandelmogelijkheden in CRPS.

Hoofdstuk 2

In tegenstelling tot warme CRPS, wordt er bij koude CRPS verondersteld dat er geen inflammatie meer aanwezig is. Er zijn echter koude CRPS-patiënten beschreven, die behandeld werden met vasodilatatoren en bij wie daarna een warme CRPS ontstond. Dit hoofdstuk bespreekt de resultaten van een retrospectief onderzoek, waarbij de hypothese 'inflammatie kan verborgen zijn door vasomotore ontregeling' werd getoetst. Hiertoe werden drie CRPS-patiëntengroepen gedefinieerd: koude, 'intermediate' (noch warm, noch koud) en warme CRPS. Van deze patiënten waren de waarden van de pro-inflammatoire cytokines IL-6 en TNF-α van de aangedane en de contralaterale zijde gemeten. Het verschil in de waarden van deze cytokines tussen de aangedane en de contralaterale zijde bleek niet significant te verschillen tussen de groepen. Deze bevinding laat zien dat er bij (een subgroep) van koude CRPS-patiënten nog sprake kan zijn van inflammatie.

Hoofdstuk 3

Auto-immuniteit is een van de mechanismen, die ten grondslag zou kunnen liggen aan de pathofysiologie van CRPS. Als bij een patiënt de verdenking bestaat op een systemische auto-immuun ziekte, wordt deze getest op de aanwezigheid van anti-nucleaire antistoffen (ANA). Anti-neuronale antistoffen zijn auto-antistoffen gericht tegen antigenen van het centrale en/of perifere zenuwstelsel. Het doel van de in dit hoofdstuk beschreven studie was om de prevalentie van deze auto-antistoffen in CRPS-patiënten te vergelijken met die van de gezonde populatie. De prevalentie van ANA bleek significant hoger te zijn in CRPS-patiënten vergeleken met de gezonde populatie; voor de anti-neuronale antistoffen gold dit echter niet. Deze bevinding geeft aan dat auto-antistoffen waarschijnlijk betrokken zijn bij de pathofysiologie van CRPS, in ieder geval bij een gedeelte van de patiënten.

Hoofdstuk 4

Immuunmodulerende medicijnen verminderen de inflammatie door beïnvloeding van de (bij de ontsteking) betrokken mediatoren. Indien inflammatie inderdaad betrokken

is bij de pathofysiologie van CRPS, dan zouden deze medicijnen een gunstig effect kunnen hebben bij CRPS-patiënten. In dit hoofdstuk wordt een overzicht gegeven van de empirische evidentie voor de effectiviteit bij CRPS van de meest gebruikte immuunmodulerende medicijnen (te weten: glucocorticoïden,TNF-α antagonisten, thalidomide, bisfosfonaten en immunoglobulines).

Hoofdstuk 5

Dit hoofdstuk presenteert de resultaten van een voortijdig beëindigd dubbelblind, placebo gecontroleerd onderzoek naar gebruik van een TNF-α antagonist (infliximab) bij CRPS. Het doel was om de (eventuele veranderingen in) klinische verschijnselen van lokale inflammatie te evalueren (door middel van de 'impairment level sumscore') na toediening van infliximab. Verder werd de normalisatie van de waarden van de mediatoren en de verbetering van kwaliteit van leven onderzocht. Er werd een trend gevonden voor een grotere daling van de TNF-α in de behandelgroep vergeleken met de placebo-groep.

Hoofdstuk 6

Mestcellen zijn betrokken bij CRPS en spelen ook een rol bij centrale sensitisatie. Bij de ontwikkeling van een meer mechanisme-georiënteerde behandeling, zou de beïnvloeding van de mestcel dan ook belangrijk kunnen zijn. Dit hoofdstuk beschrijft de 'rationale' voor het gebruik van medicijnen, die van invloed zijn op (de effecten van) mestcellen, bij de behandeling van CRPS.

Hoofdstuk 7

In de discussie wordt de focus van dit proefschrift besproken. De huidige kennis en de theorie over de rol van inflammatie in CRPS wordt samengevat. Verder worden alle bevindingen (uit gedaan onderzoek) uit dit proefschrift becommentarieerd. Dit hoofdstuk brengt ook een andere dan de vigerende conceptualisatie van de pathogenese van CRPS ter sprake: bij CRPS gaat het om continuüm van inflammatie, waarbij de verschillende vormen waarin CRPS zich kan uiten een consequentie hiervan zijn en dus niet op zichzelf staande fenotypes.

Appendices

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Ik vind dat ik een bijzondere baanconstructie heb, gedeeltelijk op het Centrum voor Pijngeneeskunde en gedeeltelijk in het Sophia kinderziekenhuis, waar ik op beide plekken met veel plezier werk. Leuk werk wordt, naar mijn idee, voor een heel belangrijk gedeelte bepaald door de mensen waarmee je werkt. Dit zal dan ook zeker hebben geholpen bij het tot een succes afronden van dit proefschrift.

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LIST OF PUBLICATIONS

Dirckx M, Schreurs MW, de Mos M, Stronks DL, Huygen FJ. The prevalence of autoantibodies in complex regional pain syndrome type 1. Mediators Inflamm 2015; 2015: 718201.

Dirckx M, Stronks DL, van Bodegraven-Hof EA, Wesseldijk F, Groeneweg JG, Huygen FJ. Inflammation in cold complex regional pain syndrome. Accepted in Acta Anaesthesiol Scand 2015

Dons-Sinke IJ, **Dirckx M**, Scoones GP. Anaesthetic management of two patients with pompe disease for caesarean section. Case Rep Anesthesiol 2014; 2014: 650310.

Dirckx M, Groeneweg G, Wesseldijk F, Stronks DL, Huygen FJ. Report of a preliminary discontinued double-blind, randomized, placebo-controlled trial of the anti-TNF-α chimeric monoclonal antibody infliximab in complex regional pain syndrome. Pain Pract 2013: 13(8): 633-40.

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Adam S, van Bommel J, Pelka M, **Dirckx M**, Jonsson D, Klein J. Propofol-induced injection pain: comparison of a modified propofol emulsion to standard propofol with premixed lidocaine. Anesth Analg 2004; 99(4): 1076-9.

CURRICULUM VITAE

Maaike Dirckx werd geboren op 6 mei 1971 te Amsterdam. Zij behaalde haar V.W.O.-diploma aan het Fons Vitae lyceum te Amsterdam. Toen zij voor de tweede keer uitlootte is ze begonnen aan de studie Medische Informatiekunde aan de Universiteit van Amsterdam (propedeuse behaald). In 1992 is zij begonnen aan de studie Geneeskunde, ook aan de Universiteit van Amsterdam. Het doctoraalexamen werd behaald in 1996 en het artsexamen werd afgerond in april 1999.

Na het afronden van haar geneeskunde opleiding heeft zij anderhalf jaar ervaring opgedaan als Eerste hulp-arts in het Sint Elisabeth Hospitaal te Curaçao; hierna volgde nog een jaar cardiologie-ervaring als assistent geneeskundige niet in opleiding (AGNIO) in het Kennemer Gasthuis te Haarlem.

In 2002 startte zij met de opleiding tot anesthesioloog in het Erasmus MC te Rotterdam (opleider: Prof.dr. J. Klein). Aansluitend is zij een jaar werkzaam geweest als fellow binnen de subspecialisaties pijngeneeskunde en kinderanesthesiologie van het Erasmus MC.

In 2008 verliet zij het Erasmus MC om als anesthesioloog in het Onze Lieve Vrouwe Gasthuis te Amsterdam te gaan werken. Sinds juni 2009 is zij weer werkzaam in het Erasmus MC en heeft zij als anesthesioloog een gecombineerde baan op de afdelingen Centrum voor Pijngeneeskunde en het Sophia Kinderziekenhuis. Sinds 2010 is zij geregistreerd in het aandachtsgebied Pijngeneeskunde en vanaf 2013 is zij tevens Medisch coördinator Pijngeneeskunde bij het Erasmus MC.

Halverwege 2010 werd gestart met het onderzoek dat tot dit proefschrift heeft geleid.

PhD PORTFOLIO

Name PhD student: Maaike Dirckx	PhD period: 2010-2015
Erasmus MC Department:	Promotor: Prof.dr. F.J.P.M. Huygen
Anesthesiology – Center for Pain Medicine	Copromotor: Dr. D.L. Stronks
Research School:	

1.PhD training	**Year**
General courses	
Research Integrity	2014
(Inter)national conferences	
6e TREND symposium, Zeist	2010
Third International Congress on Neuropathic Pain, Athens	2010
TREND symposium, Oegstgeest	2011
Pain in Europe VII, Hamburg	2011
International symposium Complex Regional Pain Syndrome, Leiden	2012
15th World Congress of Pain Clinicians, Granada	2012
Masterclass Neuropathic Pain, Rotterdam	2013
7th World Congress of the World Institute of Pain, Maastricht	2014
Other	
Persoonlijk Leiderschapsprogramma	2011
Participation in kernredactie richtlijn 'Herziening Complex Regionaal Pijn Syndroom type 1'	2012-2014
Presentation of results anti-TNF trial in international research meeting	2013
2.Teaching	**Year**
Presentation at Regionale CRPS patiëntenvereniging: CRPS en inflammatie	2012
Masterclass Vaardigheidsonderwijs ("blok pijn")	2013 + 2014